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LINKAGE RELATIONS OF THE GOLDFOIL FACTOR FOR RESISTANCE TO MILDEW IN BARLEY [1]

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INTRODUCTION

Seven different genetic factors for resistance to race 3 of mildew (*Erysiphe graminis hordei*) in barley (*Hordeum vulgare*) have been discovered in the writers' work with this disease (*7*).[2] Six of these factors have either occurred singly in varieties or have been set up in hybrid lines. Two, the Algerian factor Ml_a and the Kwan factor Ml_k, are linked (*2*). The others appear to be independent. Since all seven linkage groups have been established in barley (*6*), the linkage groups to which these seven mildew-resistant factors belong may be ascertained. Linkage relations of the Goldfoil factor are now available.

Incidental to this study, data were accumulated on a factor pair for red vs. green stem color that has not heretofore been reported. Linkage relationships were established for this factor also.

MATERIALS AND METHODS

Briggs and Barry (*1*) reported that the resistance of Goldfoil was due to a single dominant factor, Ml_g, and pointed out that this factor was independent of six-row vs. two-row and long vs. short-haired rachilla, characters available in the cross between Goldfoil and Atlas, and known to belong to linkage groups I and V, respectively. Since these findings were reported Goldfoil has been crossed with susceptible varieties carrying markers for four of the remaining groups. The cross with Nepal 595 was investigated first because it was segregating for two factor pairs each in groups III and IV. Group 3 was marked by hulled vs. naked, *Nn*, and by one of a complementary factor pair for blue vs. white aleurone, Bl_1bl_1, recently reported by Myler and Stanford (*4*). Group IV was marked by hooded vs. awned, *Kk*, and by the other complementary factor pair for blue vs. white, *Blbl*.

Goldfoil is resistant to mildew, is hulled, awned, and white, but carries the *Bl* factor for blue aleurone. Under favorable light conditions it develops red pigment in the stems. Nepal 595 is susceptible to mildew, is naked, hooded, and white, but has the Bl_1 factor for blue. In contrast to Goldfoil it has green stems.

As in previous experiments, the mildew tests were made in the greenhouse with race 3 of the fungus. Mildew classifications were based on the reaction of F_3 progeny, which were grown in greenhouse benches and which in most cases were represented by 25 to 30 plants. Every tenth row was seeded to Atlas as a check. The plants were inoculated in the three-leaf stage by dusting with spores from diseased plants grown for that purpose. The F_3 rows were classified as resistant,

[1] Received for publication March 27, 1942.

[2] Italic numbers in parentheses refer to Literature Cited, p. 5.

segregating, or susceptible. Since this factor for mildew resistant is dominant, a segregating row indicated a resistant F_2 plant and is so considered in the calculations.

When light intensity was sufficient to cause the red stem color to develop, the F_3 rows were also classified for this character. Homozygous red and segregating rows were listed as red.

The cross-over values and their probable errors were all taken from Immer's tables (*3*).

EXPERIMENTAL RESULTS

A total of 795 F_2 plants of Goldfoil×Nepal 595 were grown in the field in 1939–40 and classified at harvest time for hooded vs. awned, hulled vs. naked, and blue vs. white aleurone. Of these, 770 were tested for mildew reaction in F_3 in the greenhouse during 1940 and 1941, while 25 lines were discarded either because of insufficient seed or because the population was too much reduced by damping-off. It soon became apparent that the Goldfoil factor for mildew resistance was linked with hooded vs. awned and consequently with the blue vs. white factor pair belonging to group IV. Resistance, accordingly, was independent of the factors represented by group III. Only the data involving factors in group IV will be presented in connection with mildew resistance.

The single contrasting characters all conform to expectations on the basis of factors previously assigned to them. There were 562 hooded: 208 awned, where 577.5:192.5 were expected on the basis of a single factor (*6*). This gives a P value greater than 0.2. As pointed out above, Myler and Stanford (*4*) found the blue aleurone color to be due to complementary factors. There were 412 blue:358 white, where 433.1:336.8 were expected on the basis of the 9:7 ratio, giving a P value between 0.1 and 0.2. Finally, there were 588 mildew resistant: 183 mildew susceptible, where 578.25:192.75 were expected on the basis of a single factor, which gives a P value greater than 0.3.

Table 1 shows the data involving hooded vs. awned and resistance vs. susceptibility.

TABLE 1.—*The F_2 segregation of the characters listed from a cross of Goldfoil (kk Ml_aMl_a)×Nepal 595 (KK ml_aml_a) with expected numbers based on independence and on a cross-over value of 18.77 percent*

Characters	Observed number	Expected number on the basis of a 9:3:3:1 ratio	χ^2 on the basis of a 9:3:3:1 ratio	Expected number on the basis of a cross-over of 18.77 percent	χ^2 on the basis of a cross-over of 18.77 percent
Hooded resistant	387	433.1	4.907	391.8	0.059
Hooded susceptible	175	144.4	6.484	185.7	.616
Awned resistant	201	144.4	22.185	185.7	1.261
Awned susceptible	7	48.1	35.119	6.8	.006
Total	770	770.0	68.695	770.0	1.942
P value			Very low		>0.3

In table 1 the observed numbers for the character combinations deviate significantly from the numbers expected on the basis of the 9:3:3:1 ratio. A cross-over value of 18.77±2.33 percent was indicated by the data. The numbers expected on the basis of this value agree satisfactorily with those obtained, giving a $P>0.3$.

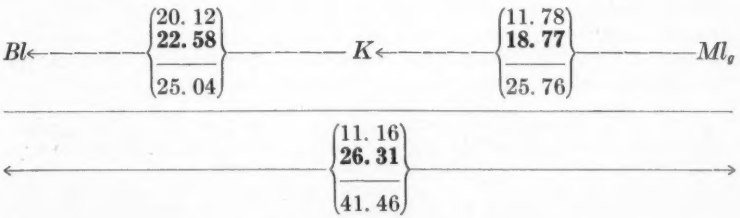
Table 2 shows the data regarding blue vs. white aleurone and resistance vs. susceptibility to mildew.

TABLE 2.—*The F_2 segregation of the characters listed from a cross of Goldfoil (Bl_1Bl_1 blbl Ml_gMl_g) × Nepal 595 (bl_1bl_1 Bl Bl ml_gml_g) with expected numbers based on independence and on 26.31 percent crossing over*

Characters	Observed number	Expected number on the basis of a 27:9:21:7 ratio	χ^2 on the basis of a 27:9:21:7 ratio	Expected number on the basis of a cross-over of 26.31 percent	χ^2 on the basis of a cross-over of 26.31 percent
Blue resistant	289	324.8	3.946	298.7	0.315
Blue susceptible	123	108.3	1.995	134.4	.967
White resistant	299	252.7	8.483	278.8	1.464
White susceptible	59	84.2	7.542	58.1	.014
Total	770	770.0	21.966	770.0	2.757
P value			Small		>.2

Robertson et al. (*5*) have shown that the *Bl* factor for blue aleurone is linked with hooded with a cross-over value of 22.58 ± 0.82. Since Myler and Stanford (*4*) have shown that this gene is present in Goldfoil, it follows that mildew resistance should show linkage with blue aleurone. Obviously the data in table 2 do not conform to independent segregation. The numbers observed agree satisfactorily, however, with those expected on the basis of 26.31 ± 5.05 percent. recombination.

The data clearly show that the Goldfoil factor pair for resistance vs. susceptibility to mildew is linked with the hooded vs. awned and the blue vs. white aleurone factor pairs, known to belong to group IV. The data are not sufficient to establish the order of these three gene pairs. The linkages reported in this paper have high probable errors because the genes entered the hybrid in the repulsion phase. The most probable cross-over values, with the maximum and minimum that might be expected based on ± 3 probable error, with the suggested order, follows:



As will be seen, the most probable value of the cross-over percentages for *Bl*—*K* and *K*—Ml_g together equals 41.35, which is just within the maximum range expected for *Bl*—Ml_g. In no other case will the sum of any two most probable values fall within the expected range of the third. The most likely order therefore seems to be *Bl*, *K*, Ml_g.

Incidental to the mildew studies, data were accumulated on red vs. green stem color, the genetics of which has not previously been reported. Goldfoil shows considerable red pigment in the stems when grown under favorable light conditions, whereas Nepal 595 produces

no red. Of the 607 plants classified for stem color, 442 were either homozygous red or segregating, while 165 were green. The numbers expected on the basis of the 3:1 ratio are 455.25:151.75, giving a probability greater than 0.2. Red stem will hereafter be designated by the symbol *Rs*.

Hulled vs. naked segregated on the basis of 3:1, which is in agreement with the results of numerous other workers (*6*). There were 448 hulled: 159 naked, where 455.25:151.75 were expected, giving a $P>0.3$. As pointed out above, blue vs. white aleurone segregated in this cross on the basis of complementary factors. The segregation of the 607 plants under consideration here agreed satisfactorily with that hypothesis, having a *P* value greater than 0.2.

It soon became apparent that red vs. green stem color was associated with hulled vs. naked. Table 3 gives the data involving these characters.

TABLE 3.—*The F_2 segregation of the characters listed from a cross of Goldfoil (NN RsRs) × Nepal 595 (nn rsrs), with expected numbers based on independent segregation and on a cross-over value of 14.5 percent*

Characters	Observed number	Expected number on the basis of a 9:3:3:1 ratio	χ^2 on the basis of a 9:3:3:1 ratio	Expected number based on a cross-over of 14.50 percent	χ^2 based on a cross-over of 14.50 percent
Hulled red	403	341.5	11.075	414.4	0.314
Hulled green	45	113.8	41.594	40.8	.432
Naked red	39	113.8	49.166	40.8	.079
Naked white	120	37.9	177.847	111.0	.730
Total	607	607.0	279.682	607.0	1.555
P value			Very small		>0.3

Obviously the observed numbers do not conform to those expected on the basis of independent segregation, but agree satisfactorily with those expected on the basis of 14.50 ± 1.06 percent crossing over. Thus the factor for red stem color belongs to linkage group III.

As Myler and Stanford (*4*) have shown, the complementary factor for blue aleurone (Bl_1) discovered by them belongs to group III and showed a cross-over percent of 9.88 ± 0.44, with naked. This gene is carried by Goldfoil. Red stem color should be linked, accordingly, with blue aleurone. That this is so may be seen from table 4.

TABLE 4.—*The F_2 segregation of the characters listed from a cross of Goldfoil (Bl_1Bl_1 RsRs blbl) × Nepal 595 (bl_1bl_1 rsrs BlBl), with expected numbers based on independent segregation and on a cross-over of 9.07 percent*

Characters	Observed number	Expected number on the basis of a 27:21:9:7 ratio	χ^2 on the basis of a 27:21:9:7 ratio	Expected number based on a cross-over of 9.07 percent	χ^2 based on a cross-over of 9.07 percent
Red blue	305	256.0	9.379	321.7	0.867
Red white	137	199.2	19.422	133.5	.092
Green blue	20	85.4	50.084	19.7	.005
Green white	145	66.4	93.042	132.1	1.260
Total	607	607.0	171.927	607.0	2.224
P value			Very small		>0.3

The expected numbers based on independent segregation deviate markedly from those observed. Those observed conform, however, to the numbers expected on the basis of 9.07 ± 1.24 percent crossing over.

Although the data are too few to indicate the order of the three gene pairs, they suggest that these genes are in the order of *N*, Bl_1, *Rs*.

SUMMARY

In a cross between Goldfoil, which carried the Goldfoil factor (Ml_g) for resistance to mildew, and which is awned and hulled, with Nepal 595, which carries the contrasting characters, it was found that mildew resistance was linked with hooded (*K*) 18.77 ± 2.33 percent, and with the *Bl* factor for blue aleurone with a cross-over of 26.31 ± 5.05 percent. As Robertson et al. (*5*) had previously shown, *K* and *Bl* are linked with a value of 22.58 ± 0.82 percent. These factors have been assigned to linkage group IV (*6*). Because the factors in this cross enter it in the repulsion phase, the probable errors are relatively high, and the order of the three genes under consideration is not clearly indicated. The order suggested is *Bl*, *K*, Ml_g.

Red stem color in this cross was found to be due to a single factor which was designated as *Rs*. This factor was found to be linked with hulled (*N*), 14.50 ± 1.06 percent, and with a second factor for blue aleurone color (Bl_1) with a cross-over value of 9.07 ± 1.24 percent. These genes have been assigned to linkage group III (*4*, *6*). Here, again, the order of the genes under consideration is not definitely indicated, but the suggested order is *N*, Bl_1, *Rs*.

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SURVIVAL, WEIGHT, AND LOCATION OF EUROPEAN CORN BORERS FEEDING ON RESISTANT AND SUSCEPTIBLE FIELD CORN[1]

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INTRODUCTION

Certain strains of corn have shown greater resistance to the survival of the European corn borer (*Pyrausta nubilalis* (Hbn.)) than have others. The utilization of this resistance in the practical control of the borer would be facilitated by a better knowledge of the factors responsible for it and of the ways in which they operate. Studies were therefore conducted in 1936 and 1937 near Toledo, Ohio, to determine, if possible, what the factors are and how, where, and when they influence the development of the borer.

One of the most resistant strains of field corn (*Zea mays* L.) yet discovered, single-cross hybrid Ill. Hy × R4, was compared with one of the most susceptible, Ill. A × Ind. TR. Although these hybrids have silked and matured their grain within 1 or 2 days of each other throughout the period of test, from 1932 to 1939, inclusive, every test has shown materially fewer borers surviving in Hy × R4 from a given number of eggs, and has indicated an inherent character for borer resistance. This character was at first thought to be delayed eclosion of the tassel, for in this hybrid the upper leaves remain wrapped around the tassel until nearly time for pollen shedding. Since the additional mature borers surviving in A × TR nearly equaled the additional immature larvae found in the tassels, it was thought probable that the availability of the tassel buds for larval feeding accounted for its relative susceptibility.

The relative survival of borers in the resistant and susceptible hybrids was studied at different stages of plant development. To determine whether the difference between the two hybrids was as great in the absence of tassels as when they are available, the tassels were removed by hand in another experiment. The age of the borers at the time the differentiation in survival occurred, the comparative weights of the borers found within the tassel buds and in other parts of the plants at different ages, and the locations in the plants of the borers throughout their growth were also studied.

EFFECT OF STAGE OF PLANT DEVELOPMENT AND PRESENCE OF TASSELS ON LARVAL SURVIVAL

To assure adequate levels of infestation and a wide range in plant development at time of borer hatching, in 1936 and 1937 corn was

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[2] The writer is indebted for assistance received from the following members of the staff of the Toledo, Ohio, laboratory to W. A. Baker under whose general supervision the work was conducted, to G. T. Bottger and Ralph Mathes for providing egg masses for the infestations, to B. A. App for supervising the counting and weighing of borers from different parts of the plants, to R. T. Everly for assembling and tabulating the data, and to Elmer Beck for the photograph in figure 3.

planted on 10 or 12 dates, and the plants were infested by hand over an extended period with egg masses produced by moths confined in cages in the laboratory.[3] Results reported in a previous publication [4] indicated that the data obtained under conditions of artificial infestation are comparable with those obtained under natural conditions.

In 1936 the experimental field was divided into 10 strips extending from north to south across the field. In these strips corn was planted, in 3-plant hills 42 inches apart each way, in successive plantings at 2- to 4-day intervals beginning on May 7, the east half of each strip to Hy × R4 and the west half to A × TR. On June 30 the field was again divided into strips at right angles to the first set, or extending from east to west. Beginning at the south end of the field, the first 4 strips of this set marked off plots of 12 hills of each hybrid in each planting and 7 other strips marked off 8-hill plots. The first strip was infested by hand on June 30 with 4 egg masses per plant, hatching within a day, in addition to a light natural infestation. Ten other infestations were made successively in strips toward the north, the last one on July 24.

The stage of plant development, as measured by the number of days from borer hatching to pollen shedding, the average number of mature or nearly mature borers per plant counted from three or four plots for each hybrid, and their ratios, expressed as percentages of the borer-susceptible hybrid, are given in table 1. In figure 1 these percentages have been plotted against the number of days between borer hatching and pollen shedding, and a freehand curve has been fitted to the data.

TABLE 1.—*Effect of stage of plant development, as measured by the interval from borer hatching to pollen shedding, on the number of mature European corn borers in resistant (Hy × R4) and susceptible (A × TR) single-cross hybrid field corn, Toledo, Ohio, 1936*

Days from borer hatching to pollen shedding	Borers per plant—			Days from borer hatching to pollen shedding	Borers per plant—		
	In A × TR	In Hy × R4	Ratio Hy × R4 to A × TR		In A × TR	In Hy × R4	Ratio Hy × R4 to A × TR
	Number	*Number*	*Percent*		*Number*	*Number*	*Percent*
38	8.2	2.5	30	17	4.7	1.5	32
35	8.7	2.6	30	16	6.8	2.5	37
33	9.6	2.5	26	16	8.5	3.5	41
31	8.0	3.5	44	14	6.0	4.1	68
29	8.3	3.3	40	14	12.3	4.7	38
28	8.4	4.1	49	13	11.2	5.8	52
26	7.5	3.1	41	11	14.7	8.1	55
26	6.6	4.0	61	11	15.5	7.9	51
25	5.9	2.5	42	10	10.9	5.8	53
23	8.7	4.3	49	9	10.0	7.7	77
23	4.9	2.5	51	9	18.4	10.3	56
22	8.9	4.5	51	7	14.0	9.2	66
21	4.8	1.6	33	6	23.1	14.6	63
21	7.5	3.4	45	5	16.3	12.4	76
18	6.2	2.2	35	4	19.3	15.6	81
18	8.3	4.6	55	2	25.7	21.3	83
17	10.7	4.8	45				

In both hybrids the number of mature borers per plant on plants on which the borers hatched within 14 days of pollen shedding showed a

[3] PATCH, L. H., and PEIRCE, L. L. LABORATORY PRODUCTION OF CLUSTERS OF EUROPEAN CORN BORER EGGS FOR USE IN HAND INFESTATION OF CORN. Jour. Econ. Ent. 26: 196-204, illus. 1933.

[4] PATCH, L. H. RESISTANCE OF A SINGLE-CROSS HYBRID STRAIN OF FIELD CORN TO EUROPEAN CORN BORER. Jour. Econ. Ent. 30: 271-278, illus. 1937.

pronounced increase over the number on plants in an earlier stage of development (table 1).

Figure 1 shows a change also in the ratio between the two hybrids on plants at this stage of development. The ratio increased gradually from 38 to 48 percent as the time between borer hatching and pollen shedding decreased from 38 to 14 days and then increased sharply to 86 percent as the interval decreased to 2 days. The increase in the ratio from 38 to 86 percent shows that hybrid Hy × R4 was much less resistant, relative to A × TR, when the borers hatched just before pollen shedding than when they hatched about 30 days before.

The period within 14 days of pollen shedding had such marked effects on borer survival and the relative number of borers in the two hybrids that the development of the plants within this period was given special study. The tassels of hybrid A × TR came into view

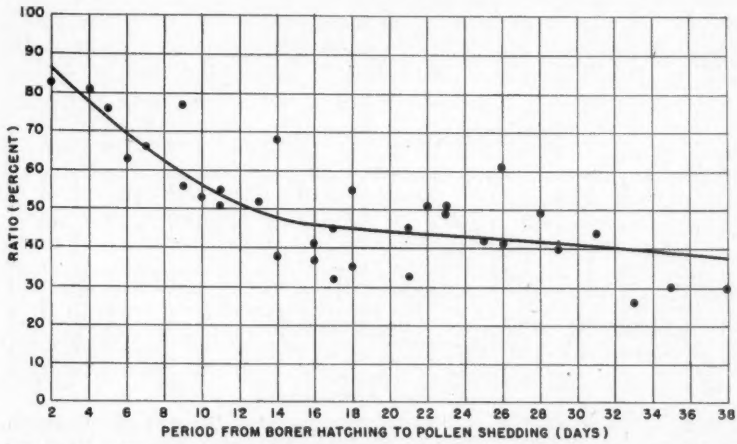

FIGURE 1.—Effect of stage of plant development on the ratio of the number of European corn borers in borer-resistant single-cross hybrid field corn Hy × R4 to the number in borer-susceptible hybrid A × TR, Toledo, Ohio, 1936.

10.3 days before they shed pollen, and the tassels of Hy × R4, although only 1.5 days later in development, came into view 4.2 days before they shed pollen. The tassels, growing up within the enveloping whorl of leaves, were undoubtedly available to the young borers before they came within view of the observer. Data from an experiment in 1937 show that in A × TR 50.5 percent of the borers, 4 days old, were in the tassels 14 to 17 days before the plants shed pollen as compared with 5.1 percent in Hy × R4. Hence the maximum availability of the tassel buds for feeding occurred, at least partly in Hy × R4 and probably wholly in A × TR, within the period when the egg infestations were made that resulted in large increases in the number of mature borers.

To determine whether increases in the level of borers in the two hybrids, both actually and relatively, would occur in the absence of tassels as well as in their presence, 9 pairs of 48-plant plots were planted on May 7, 1937, one plot of each pair to Hy × R4 and the

other to A × TR. All plots were infested with 3 egg masses per plant. On the first 3 pairs of plots the borers hatched, respectively, on July 4, 8, and 12, when the tassels were not visible to the observer, and on the other 6 pairs the borers hatched between July 16, when most of the tassels of A × TR were just visible, and July 24, when many tassels of A × TR were shedding pollen. Before the eggs were placed on the plants of these 6 pairs, the tassels were pulled from one-half of each plot, except the July 16 pair of plots, from which they could not be pulled without mutilating the plants.

The number of borers per plant in each hybrid from the detasseled plants in the plots where the borers hatched on July 19, 20, 21, 23, and 24 were plotted against the date of borer hatch, and freehand curves were fitted to these data (fig. 2). The curve for A × TR is shown as a broken line between July 8 and 19, because the plotted data from the plots where the borers hatched on July 12 and 16 were not used in fitting the curve. The number of borers in the plot where the borers hatched on July 16 was considerably above the level expected from the broken curve in figure 2, because the tassels could not

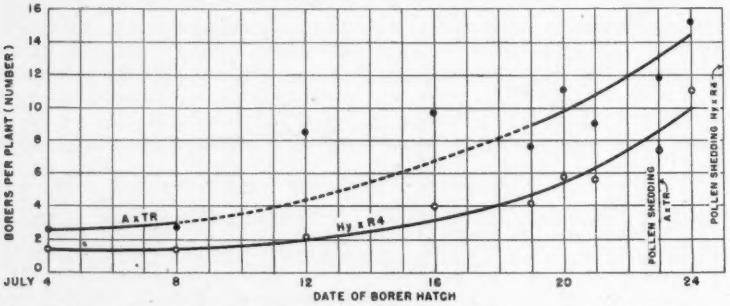

FIGURE 2.—Effect of stage of plant development on the level of the European corn borer population in borer-resistant single-cross hybrid field corn Hy × R4 and borer-susceptible hybrid A × TR when the effect of the tassels was eliminated, Toledo, Ohio, 1937.

be removed. The tassels in the plot of A × TR where the borers hatched on July 12 were beginning to show on July 13. Since 50.5 percent of the borers 4 days old were in tassel buds from 4 to 7 days before the tassels became visible to the observer, the higher-than-expected level of borers in the July 12 plot of A × TR was undoubtedly due to the availability of the tassels.

The trend of the curves in figure 2 indicates that the levels of borers were higher on the more mature plants of both hybrids even though the effect of the tassels had been eliminated. The effect of weather on borer establishment was a possible factor. Records taken in the field show that the weather was especially uniform between July 18 and 25, when the detasseled plants were infested. Between these dates no rain fell and the temperature did not rise above 90° F. A heavy rain of 2.22 inches fell on July 25. However, since there were more borers in the plots of both hybrids where the borers hatched on July 24 than in the other plots, rainfall had no inhibiting effect on borer establishment. The weather between July 3 and 13, when the

infestations were made that resulted in low borer populations, is believed to have been favorable for borer establishment. Stirrett,[5] in studying the effect of four or five rainstorms during the hatching period in each of 3 years, concluded that rain has little effect on larval survival. It is probable, therefore, that the higher borer survival on the more mature plants, indicated in figure 2, was not the result of weather conditions.

From the data at hand the conclusion is drawn that the rate of borer survival increases with advance in plant development at time of borer hatch, at least up to the pollen-shedding stage, and that there is an additional rise in the borer level in those hybrids in which the tassels are more accessible to the young borers. Hybrids with delayed tassel eclosion would probably partly inhibit borer survival if the borers hatched within 14 days of pollen shedding.

The curves in figure 2 indicate that there was an increasingly larger percentage of borers in Hy × R4 as compared with A × TR as the plants approached the pollen-shedding stage. Data taken from the curves show that in the plants where borers hatched July 8 an average of 50.0 percent as many borers were in Hy × R4 as in A × TR, as compared with 52.3 and 68.1 percent in the detasseled plants where borers hatched on July 19 and 24, respectively. Hence it appears that hybrid Hy × R4 lost some of its resistance to the borer relative to A × TR when the borers hatched on plants approaching close to the pollen-shedding stage, even though the effect of the tassels had been removed.

AGE OF BORERS AT TIME OF DIFFERENTIATION IN SURVIVAL

Another experiment similar to that of 1936 was conducted in 1937, which comprised 12 plantings of A × TR and Hy × R4 from May 7 to June 5. Manual infestations with eggs hatching within a day were made on July 8 and 12 on separate plots of each planting. Soon after the eggs hatched, two plants of each hybrid were taken from each planting each work day, the borers in them counted, and their locations in the plants noted. Dissection of plants infested on July 8 alternated with that of plants infested on July 12. It was possible to combine the derived data so that 12-plant samples were made up for 12 different ages of the borers ranging from 4 to 55 days for groups of plants shedding pollen 14, 17, 18, 20, 21, 24, 28, and 32 days after borer hatching. These data were further combined by averaging the 14-, 17-, and 18-day groups from plants in a late stage of development, the 20- and 21-day groups from plants in a midseason stage, and the 24-, 28-, and 32-day groups from plants in an early stage. As a result samples of 36 or 24 plants and a somewhat stabilized ratio of the number of borers in Hy × R4 to those in A × TR were obtained (table 2).

The numbers of borers in the various age groups are given in table 2. In hybrid A × TR the population of borers in the 50.0-day age group of plants in the late stage of development was 58.4 percent, and in plants in the midseason stage 29.1 percent, greater than in plants in

[5] STIRRETT, G. M. A FIELD STUDY OF THE FLIGHT, OVIPOSITION AND ESTABLISHMENT PERIODS IN THE LIFE CYCLE OF THE EUROPEAN CORN BORER-PYRAUSTA NUBILALIS, HBN., AND THE PHYSICAL FACTORS AFFECTING THEM. V. THE SEASONAL CHARACTERISTICS OF FLIGHT, OVIPOSITION AND LARVAL ESTABLISHMENT. THE VARIATIONS AND EFFECTS OF SEASONAL CLIMATE. THE FACTORS CAUSING FLUCTUATIONS IN BORER POPULATIONS. Sci. Agr. 18: 655-683, illus. 1938.

the early stage of development. In the hybrid Hy × R4 there were 39.9 and 21.0 percent more, respectively, in plants in the last two stages of development. Apparently, conditions for borer survival did not improve so much in Hy × R4 as they did in A × TR, especially in plants in the late stage of development, for there were 39 and 42 percent as many borers in Hy × R4 as in A × TR in the late and midseason stages, respectively, as compared with 45 percent in the early stage. In the 1936 experiment Hy × R4 contained about 46 percent as many mature borers as did A × TR in plants of the early stage, and this percentage increased to 86 (fig. 1) as the time from borer hatching to pollen shedding decreased from 14 days to 2 days.

TABLE 2.—*European corn borers of different ages infesting borer-resistant (Hy × R4) and borer-susceptible (A × TR) single-cross hybrid field corn in different stages of development at time of borer hatching, Toledo, Ohio, 1937*

Age of borers (days)	Borers per plant—(plants in early stage)			Borers per plant—(plants in midseason stage)			Borers per plant—(plants in late stage)		
	In A × TR	In Hy × R4	Ratio Hy × R4 to A × TR	In A × TR	In Hy × R4	Ratio Hy × R4 to A × TR	In A × TR	In Hy × R4	Ratio Hy × R4 to A × TR
	Number	*Number*	*Percent*	*Number*	*Number*	*Percent*	*Number*	*Number*	*Percent*
4 [1]	11.5	6.4	56	15.9	9.2	58	17.7	11.5	65
8	8.8	3.6	.....	10.0	5.0	.....	10.3	3.7	.....
13	5.9	2.6	.....	6.3	4.0	.....	9.8	3.0	.....
17	3.9	2.4	.....	6.5	3.0	.....	8.3	3.6	.....
22	3.0	2.1	.....	6.6	2.4	.....	7.1	2.6	.....
Mean: 15.0	5.40	2.68	50	7.35	3.60	49	8.88	3.23	36
26	3.9	2.4	.....	4.5	2.6	.....	6.8	2.8	.....
31	3.5	2.1	.....	4.1	2.2	.....	5.6	2.0	.....
36	3.1	2.1	.....	4.3	1.6	.....	5.2	2.4	.....
41	2.6	1.2	.....	5.1	2.4	.....	5.6	2.4	.....
Mean: 33.5	3.28	1.95	59	4.50	2.20	49	5.80	2.40	41
45	3.0	1.3	.....	4.9	2.0	.....	6.1	2.1	.....
50	3.0	1.6	.....	3.7	1.6	.....	5.1	2.1	.....
55	3.6	1.4	.....	3.8	1.6	.....	4.0	1.8	.....
Mean: 50.0	3.20	1.43	45	4.13	1.73	42	5.07	2.00	39

[1] Data for borers 4 days old not included in calculating means for the age group 8 to 22 days.

Table 2 also shows that the borer population in both hybrids decreased considerably with advancing borer age, most of the decrease in Hy × R4 occurring before the eighth day. The ratio of the number of borers in Hy × R4 to the number in A × TR, however, decreased little after the fourth day. At 4 days old from 56 to 65 percent as many borers were found in Hy × R4 as in A × TR, depending on the stage of the plants, and with borers averaging 50.0 days old the percentages ranged from 45 to 39, but it is doubtful whether these percentages differ significantly from those based on the two preceding age groups. Most of the differentiation in the borer population between the two hybrids therefore occurred before the borers were 4 days old.

More detailed data from the plants infested on July 8 give totals of 506, 587, and 343 young larvae in 24 plants of A × TR on the first, second, and fourth days after hatching. Samples of Hy × R4 contained 88, 55, and 49 percent as many larvae, respectively, showing

again that the differentiation in the number of borers in the two hybrids occurred within the first 4 days after hatching.

COMPARATIVE WEIGHTS OF BORERS AT DIFFERENT AGES

At various times during the first 57 days of larval life borers were dissected from the sample plants and weighed on the day of dissection. In the course of dissection many borers were accidentally cut and were not weighed. All the borers taken from the tassels of Hy × R4 and available for weighing totaled less than 100, and no comparisons were possible between these borers and borers from other locations. The samples of borers from the tassels of A × TR, however, were adequate, and the weight of borers of the same age averaged more than that of borers taken from other parts of the plant. When 7.4, 13.5, 19.1, and 24.6 days old, borers from parts of plants other than the tassels weighed 69.8, 82.4, 81.1, and 76.3 percent as much, respectively, as borers taken from the tassel buds or tassel stalks. The young borers up to 25 days old evidently found the tassels more nutritious than other parts of the plant.

Comparisons were made between the weights of borers taken from the entire plants of A × TR and Hy × R4. The growth curve of the borers from the 4 latest plantings of Hy × R4 did not differ essentially from that of the borers from the 4 earliest plantings. The borers from the 4 earliest plantings of A × TR weighed more than the borers from the 4 latest plantings, probably in part because 39 percent were from the tassels in the 4 earliest plantings as compared with 12 percent in the 4 latest plantings. Table 3 gives the number of borers and their mean weights from the 4 earliest and 4 latest plantings of A × TR and from these plantings combined in the case of Hy × R4. A sample of 200 newly hatched borers averaged 0.11 mg. per borer, but not until the borers were 12 days old were weighings of borers from all plantings made.

TABLE 3.—*Weights of European corn borer larvae of different ages taken from the entire plants of borer-resistant (Hy × R4) and borer-susceptible (A × TR) single-cross hybrid field corn, Toledo, Ohio, 1937*

Mean age of borers (days)	A × TR				Hy × R4		Ratio of weight of borers in Hy × R4 to those in A × TR[1]	
	4 earliest plantings		4 latest plantings		Total of 4 earliest and 4 latest plantings			
	Borers	Mean weight	Borers	Mean weight	Borers	Mean weight	4 earliest plantings	4 latest plantings
	Number	*Milligrams*	*Number*	*Milligrams*	*Number*	*Milligrams*	*Percent*	*Percent*
13.6	260	7.0	148	5.2	136	5.6	80.0	107.7
18.4	230	10.8	60	8.2	144	7.0	64.8	85.4
23.1	191	24.5	76	17.2	116	15.9	64.9	92.4
27.8	167	42.9	102	33.2	105	29.6	69.0	89.2
32.5	161	71.7	59	51.4	94	36.3	50.6	70.6
37.2	143	93.9	65	88.3	108	51.8	55.2	58.7
41.7	179	105.1	69	99.7	97	83.0	79.0	83.2
46.4	151	121.7	62	108.9	90	93.1	76.5	85.5
51.2	130	113.5	74	119.6	99	94.4	83.2	78.9
56.8	210	122.0	114	116.1	118	110.8	90.8	95.4

[1] Data for Hy × R4 are based upon the total of the 4 earliest plantings and the 4 latest plantings.

The ratios of weights of borers in Hy × R4 relative to those in A × TR decreased with advancing age until about 33 to 37 days

after hatching and then increased as the borers approached maturity. This relationship indicates that during the first half of the growth period the borers in A × TR grew faster than those in Hy × R4, but during the last half of the period they approached their full-fed weight

FIGURE 3.—Two types of feeding areas of the European corn borer on the leaf tissue of field corn: *A*, Borer-susceptible single-cross hybrid A × TR; *B*, borer-resistant Hy × R4.

relatively more slowly, whereas the borers in Hy × R4 gained enough more in weight during the last half of the growth period partly to overcome their under size during the first half. When 56.8 days old the borers in Hy × R4 weighed more than 90 percent as much as the borers in A × TR.

Averages of the ratios in table 3 show that from 18.4 to 46.4 days after hatching the borers in Hy × R4 weighed 65.7 percent as much as those in the first four plantings, and 80.7 percent as much as those in the last four plantings of A × TR. From the data in table 2 it may be seen that during the period 17 to 45 days after the hatching of a given number of eggs the survival on Hy × R4 averaged 48 percent of the survival on A × TR. Thus, since both the weight of the surviving borers and the percentage of survival were lower in Hy × R4 than in A × TR, it is evident that the former is a less suitable food for the borers.

In the preceding section it was shown that the earlier plantings provided better conditions for the survival of borers in both hybrids, especially in A × TR (table 2). A comparison of the weight ratios of 65.7 and 80.7 percent, coupled with the fact that the growth curves for larvae in the four earliest and four latest plantings of Hy × R4 were the same, indicates that the borers in A × TR gained more in growth over the borers in Hy × R4 in the earliest plantings than in the latest plantings. Hence, the borers in the earliest plantings of A × TR compared with borers in the latest plantings encountered better conditions for survival and also for a more rapid growth than did the borers in corresponding plantings of Hy × R4.

The feeding responses of newly hatched larvae on the leaf tissue of Hy × R4 also indicate that this hybrid is imperfectly adapted to them. The pin-point feeding areas on Hy × R4, as shown in figure 3, suggest that the young larvae made numerous attempts to find suitable food, whereas the narrow, elongated feeding areas on A × TR suggest that the larvae found suitable food on the first trial and remained to complete feeding. Bottger's [6] observations further suggest that the fine-textured excrement, greatly changed in color, from borers feeding on internodes of A × TR indicates a more nearly complete digestion than does the coarse-tectured excrement, retaining the original color of the food, from borers in Hy × R4.

LOCATION OF BORERS IN THE PLANTS

The basic data summarized for table 2 were combined further to learn the feeding locations in the two hybrids at different ages of borers. Table 4 gives the percentages of the total number of borers feeding in different locations for four age groups and for four groups of plants differing in stage of development. Since it has been shown in table 2 that about the same percentage of borers died with advancing age in Hy × R4 as in A × TR beyond the fourth day after hatching, a direct comparison may be made between the percentages of the borers in the different locations in the two hybrids.

The first marked difference observed in the location of the borers was in their distribution between the tassels and whorls up to the time they were 13 days old. The larger percentage found in the tassels of some plants was offset by a nearly equal larger percentage in the whorls of other plants. This difference in location occurred both between plants of A × TR and Hy × R4 in nearly the same stage of development at time of borer hatch but with a delayed eclosion of the tassels in Hy × R4 and between plants of A × TR in a relatively

[6] BOTTGER, G. T. PRELIMINARY STUDIES OF THE NUTRITIVE REQUIREMENTS OF THE EUROPEAN CORN BORER Jour. Agr. Res. 60: 249-258. 1940.

late stage and plants in an early stage with undeveloped tassels. When borers 4 to 13 days old hatched from 14 to 20 days before pollen shedding, averages of 39.2 and 25.1 percent were in the tassels and whorls of A × TR, respectively, as compared with 7.4 and 51.2 percent in Hy × R4. The 31.8 larger percentage of borers in the tassels of A × TR does not differ greatly from the 26.1 larger percentage in the whorls of Hy × R4. When borers of the same age hatched from 21 to 32 days before pollen shedding, averages of 6.5 and 64.4 percent were in the tassels and whorls, respectively, of A × TR. The 32.7 larger percentage in the tassels of the relatively more mature plants of A × TR does not differ greatly from the 39.3 larger percentage of borers in the whorls of the less mature plants of A × TR.

TABLE 4.—*Percentage of the total number of European corn borers of different ages located in the parts of plants of borer-resistant (Hy × R4) as compared with borer-susceptible (A × TR) single-cross hybrid field corn when the borers hatched 14 to 32 days before the plants shed pollen, Toledo, Ohio, 1937*

Age of borers (days)	Interval between borer hatching and pollen shedding	Total borers in 72 plants		Borers found—																	
				Within tassel buds or stalks		On leaf										Within ear buds or ears		Within internodes of stalk	
						Within whorl		Under ligules		Within midrib		On surface		Behind sheaths					
		Hy × R4	A × TR	Hy × R4	A × TR	Hy × R4	A × TR	Hy × R4	A × TR	Hy × R4	A × TR	Hy × R4	A × TR	Hy × R4	A × TR	Hy × R4	A × TR	Hy × R4	A × TR
	Days	*No.*	*No.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
4–13	14–17	492	1,068	12.0	53.8	44.0	13.4	28.1	19.0	4.3	1.8	2.5	1.3	7.2	6.8	1.9	1.7	0	2.2
	18–20	409	612	2.7	24.6	58.3	36.7	23.5	23.6	1.9	3.8	3.0	2.8	10.1	7.5	.5	.2	0	.8
	21–24	358	920	1.2	11.9	52.1	55.3	25.5	12.9	4.2	1.9	6.5	1.4	10.2	16.6	.3	0	0	0
	28–32	289	475	0	1.1	59.9	73.4	13.1	14.9	6.5	1.5	12.8	2.6	6.9	6.5	.8	0	0	0
	Mean	----	----	4.0	22.9	53.6	44.7	22.5	17.6	4.2	2.3	6.2	2.0	8.6	9.3	.9	.5	0	.7
17–26	14–17	266	635	13.5	19.9	1.3	.5	35.8	27.7	9.5	3.1	2.8	.8	13.8	14.6	9.7	17.7	13.6	15.7
	18–20	150	352	14.2	24.6	6.3	1.4	44.1	33.7	10.7	5.1	2.5	2.3	9.4	9.8	2.4	10.1	10.4	13.0
	21–24	223	436	11.0	23.7	9.3	2.1	40.6	30.9	15.9	10.3	1.4	2.1	10.0	13.5	.9	3.1	10.9	14.3
	28–32	127	185	4.8	10.8	16.2	16.1	42.9	37.1	16.7	9.3	2.9	1.5	3.3	8.6	3.7	2.3	9.5	14.3
	Mean	----	----	10.9	19.8	8.3	5.0	40.8	32.4	13.2	6.9	2.4	1.7	9.1	11.6	4.2	8.3	11.1	14.3
31–41	14–17	188	485	5.6	5.8	----	----	11.2	4.0	1.9	.2	1.1	0	13.1	8.0	26.4	30.9	40.7	51.1
	18–20	119	246	7.9	11.2	----	----	13.7	7.8	1.5	3.4	1.5	.5	19.2	10.6	14.8	23.9	41.4	42.6
	21–24	176	349	6.9	10.8	----	----	17.6	10.8	7.9	.9	1.3	.5	19.0	11.1	13.6	20.8	33.7	45.1
	28–32	103	159	10.7	10.7	----	----	16.5	10.1	4.4	1.3	1.7	1.2	12.1	10.0	5.1	11.9	49.5	54.8
	Mean	----	----	7.8	9.6	----	----	14.8	8.2	3.9	1.5	1.4	.5	15.8	9.9	15.0	21.9	41.3	48.4
45–55	14–17	156	424	1.9	1.9	----	----	2.0	0	0	0	.6	.2	18.8	13.3	20.3	17.5	56.4	67.1
	18–20	122	266	1.6	1.8	----	----	.7	.3	.7	.3	0	.8	25.6	16.8	17.4	11.8	54.0	68.2
	21–24	142	355	2.9	3.6	----	----	0	.6	0	0	1.5	.6	18.2	9.1	11.3	18.2	66.1	67.9
	28–32	74	142	2.6	2.9	----	----	4.4	.8	1.2	0	0	0	20.6	15.8	8.9	10.4	62.3	70.1
	Mean	----	----	2.2	2.5	----	----	1.8	.4	.5	.1	.5	.4	20.8	13.8	14.5	14.5	59.7	68.3

It was shown previously that the differentiation in the number of borers found in the two hybrids occurred before the borers were 4 days old. It was indicated also that 50.5 and 5.1 percent of the borers 4 days old were in the tassels of A × TR and Hy × R4, respectively, 14 to 17 days before the plants shed pollen. In other samples of plants of the same plantings, but infested earlier when the tassels were not available, during the first 4 days of larval life 662, or 70 percent, of 943 borers were within the whorl of leaves of Hy × R4, and 1,107, or 77 percent, of 1,436 borers were within the whorl of leaves of A × TR. Therefore, most of the differentiation in the

numbers of borers surviving in Hy × R4 and A × TR occurred in this part of the plant.

The percentage of borers of all ages in the tassels and the total percentage in the ears and internodes were larger in A × TR, but the total percentage in the whorls, ligules, and midribs was larger in Hy × R4. These differences are best shown in table 5, where the data from table 4 have been consolidated for two stages of the plants and four age groups of borers.

TABLE 5.—*Location of European corn borers of different ages in plants of borer-resistant (Hy × R4) and borer-susceptible (A × TR) single-cross hybrid field corn, Toledo, Ohio, 1937. Consolidation of data in table 4*

Days between borer hatching and pollen shedding	Age of borers	Borers found—							
		In tassels		In whorls, ligules, and midribs of leaves		In ear buds or ears and internodes of stalk		On leaf surface and behind leaf sheaths	
		Hy × R4	A × TR	Hy × R4	A × TR	Hy × R4	A × TR	Hy × R4	A × TR
	Days	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
14–20	4–13	7.4	39.2	80.0	49.2	1.2	2.5	11.4	9.2
	17–26	13.9	22.3	53.9	35.8	18.0	28.3	14.3	13.8
	31–41	6.8	8.5	14.2	7.7	61.7	74.3	17.5	9.6
	45–55	1.8	1.9	1.7	.3	74.0	82.3	22.5	15.5
21–32	4–13	.6	6.5	80.7	80.0	.6	0	18.2	13.6
	17–26	7.9	17.3	70.8	52.9	12.5	17.0	8.8	12.9
	31–41	8.8	10.8	23.2	11.6	51.0	66.3	17.0	11.4
	45–55	2.8	3.3	2.8	.7	74.3	83.3	20.1	12.8

For all age groups except one there was little difference between groups in the percentage of the borers in the tassels. For the borers in the first age group in plants in the later stage of development the percentage in the tassels of Hy × R4 was 31.8 less than in those of A × TR. Borers of the next age group showed a decrease in the percentage in the tassels of A × TR and an increase in those of Hy × R4. The delay in the eclosion of the tassels of Hy × R4 and the resulting greater inaccessibility of the tassel buds to the young larvae is the only known reason for the smaller percentages of borers in the tassels of that hybrid.

For the borers in the first age group in plants in the later stage of development there were 49.2 percent in the whorls, ligules, and midribs of A × TR as compared with 80.0 percent in those of Hy × R4. The difference, which was almost equal to the difference in the percentages in the tassels but reversed, showed the effect of the delayed tassel eclosion of Hy × R4 in keeping the young larvae from entering the tassels.

In plants in the early stage of development the two hybrids showed nearly equal total percentages of borers of the earliest age group in the whorls, ligules, and midribs, and most of the remaining borers were on the surface of leaves and behind leaf sheaths. At this stage the tassels in both hybrids were mostly undeveloped and only relatively few borers became established in them.

As the borers grew older they left the whorls, ligules, and midribs to go into the ear buds, or ears, and internodes. Some of the borers in the tassel buds entered the tassel stalks, and many went to other locations, finding eventual establishment in the ears and internodes.

For all age groups, when more than 1 percent of the borers is considered, from about 5 to 15 percent more borers were in the ears and internodes of A × TR than of Hy × R4, an indication that they went to these locations earlier in A × TR. Consequently there were larger percentages in the whorl, ligules, and midribs of Hy × R4. The larger percentages of borers in the ears and internodes of A × TR were possibly associated with the larger size of the borers in this hybrid, the larger borers migrating to these parts before they did in Hy × R4.

For borers up to 26 days old the differences between the two hybrids in the percentages found on the surface of leaves and behind leaf sheaths are of minor consideration. The average for these borers in both plant-development groups was 13.2 percent in the case of Hy × R4 and 12.3 percent in the case of A × TR (table 5). For borers 31 to 55 days old on an average less than 1.5 per cent of the borers were on leaf surfaces in either hybrid (table 4). However, on an average 18.3 percent were behind the leaf sheaths in Hy × R4 as compared with 11.9 percent in A × TR.

The larger percentage behind the leaf sheaths of Hy × R4 was, again, probably a manifestation of the smaller size of the borers in that hybrid and of a resulting delay in entering the ears and internodes. The total percentage in the ears, internodes, and tassel stalks averaged 82.7 in A × TR as compared with 70.3 in Hy × R4. Hence, the smaller percentage in these locations in Hy × R4 indicates an equal larger percentage in other locations as compared with A × TR. Of the 12.4 larger percentage in other locations of Hy × R4, 5.4 percent were located at the ligules of the leaves and in the midribs and 6.4 percent were behind the leaf sheaths. Probably most of the borers behind the leaf sheaths and at the adjoining ligules were just about to bore into the internodes. Many of the borers were seen boring into the internodes when the plants were dissected.

To complete the data on the parts of the plants infested, the percentages of borers at 26 and 55 days old occurring in particular internodes were determined. Between these ages the relationship between percentages of borers and ages of borers in the internodes was found to be linear. A few borers were located in the thirteenth internode of both hybrids, or the internode just below the tassel. In the twelfth to ninth internodes, called the top third of the internodes, as the age of the borers increased 29 days, the 33 percent decreased to 14 in A × TR and 35 percent decreased to 25 in Hy × R4. In the eighth to fifth internodes 42 percent increased to 54 in A × TR and 41 percent remained constant in Hy × R4. In the lowest third of the internodes, or the 4 internodes above the surface of the ground, 19 percent increased to 28 in A × TR and 8 percent increased to 29 in Hy × R4. These data show that when the borers were about half grown nearly the same percentage were in the top third of the internodes of both hybrids and a smaller percentage were in the lowest third of the internodes of the resistant hybrid. With increasing age the borers left the top third of the internodes to enter those lower down, but they did so less extensively in the resistant hybrid.

SUMMARY

The discovery that a much smaller number of European corn borers matured from a given number of eggs hatching on resistant than on

susceptible field corn suggested that delayed eclosion of the tassel might be an important factor in borer survival. A study was therefore made of the effect of the stage of plant development and the presence of tassels at time of borer hatching on two single-cross hybrids, borer-resistant Ill. Hy × R4 and borer-susceptible Ill. A × Ind. TR. The comparative numbers and weights of the larvae from hatching to maturity and their location in the plants after the differentiation in numbers occurred were also studied.

Most of the differentiation in numbers of borers occurred within the first few days after hatching; thereafter survival was relatively the same in both hybrids.

The borers developed at a slower rate in the resistant than in the susceptible hybrid. Feeding characteristics as well as weights of borers showed the resistant hybrid to be less suitable as food.

The greater availability of the tassel buds in the susceptible hybrid seems to contribute to more rapid growth of the borers, but general unsuitability of the resistant hybrid for food is evidently a major factor in survival and probably in slower growth.

Differences in borer survival between the resistant and susceptible hybrid became less as the plants were nearer the pollen-shedding stage at time of borer hatching, both when detasseled and normal plants were used.

In plants shedding pollen 14 to 20 days after borer hatch, a much larger percentage of borers less than 13 days old were in the tassels of the susceptible hybrid, but almost an equally larger percentage were in the whorls, ligules, and midribs of the resistant hybrid. Most of the differentiation in survival occurred in the whorl of leaves. Larger percentages of the borers 17 to 55 days old were in the ears and internodes of the susceptible than of the resistant hybrid. When the borers were about half grown, nearly the same percentage were in the top third of the internodes of both hybrids and a smaller percentage in the lowest third of the internodes of the resistant hybrid. With increasing age the borers left the top third of the internodes to enter the internodes lower down, but they did so less extensively in the resistant hybrid.

A PYTHIUM STALK ROT OF CORN [1]

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INTRODUCTION

A stalk rot, later shown to be caused by a species of *Pythium*, was first observed by M. T. Jenkins [2] during late July and early August 1940, on the Potomac River bottoms of the Arlington Experiment Farm, Arlington, Va., on plants of two inbred lines of dent corn (*Zea mays* L.). All 17 plants in a row of K167, an inbred line developed at the Kansas Agricultural Experiment Station, were infected and, when 15 to 20 inches high, had fallen over just above the ground. The top of each plant could easily be lifted from the crown and root. Adjacent rows of K168 showed no infection. A few days after the discovery of the disease a similar stalk rot was observed in the same nursery in six rows of C. I.[3] 6, an inbred line developed by the Division of Cereal Crops and Diseases at the Arlington Farm. These plants were 5 feet tall and had stalks 1 inch or more in diameter. The six rows alternated with other inbreds, but infection occurred only in C. I. 6. A few plants in each of the six rows fell over, owing to rotting of the first or second internode above the soil line. In most cases the tops remained green and turgid for a week or more. Both infections appeared after a heavy rain and a subsequent period of very high temperature and high humidity. The infection did not spread and when the temperature went down appeared to cease as suddenly as it had developed.

THE DISEASE

The pythium stalk rot of corn considered here develops rapidly during a few days of very high temperature and high humidity, becomes apparent when the plants fall over, and then, when the temperature falls, progresses no farther. The fungus destroys the stalk tissue for 1 or 2 inches at one of the lower internodes, causing large plants to fall over, and then, instead of progressing to new tissue, apparently ceases its activity. The roots are abundant and healthy. There is no stunting of the plants. They are healthy and grow vigorously, and the disease becomes apparent only when the stalks bend abruptly at one of the lower internodes and fall to the ground (fig. 1). The outer rind of the cornstalk, the epidermis, the lignified cells of the hypodermis, and the inner parenchyma cells are softened, disorganized, and destroyed, leaving only the separate browned strands of vascular bundles, which cannot support the plant (fig. 2).

[1] Received for publication April 2, 1942.

[2] Principal agronomist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

[3] C. I. refers to accession number of the Division of Cereal Crops and Diseases. Formerly, accession numbers for the inbred lines developed in this Division were designated by the prefix "U. S." instead of "C. I." but have been changed recently to avoid confusion with the designation of U. S. hybrids.

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FIGURE 1.—Pythium stalk rot in inbred lines of yellow dent corn. Natural infection in the field at the Arlington Experiment Farm, Arlington, Va. Photographed by M. T. Jenkins. *A*, Row 1,231, C. I. 6. Photographed August 8, 1940. *B*, Inbred lines: *a*, K168; *b*, K167; *c*, K168. Photographed July 29, 1940.

In the lignified tissue of the older plants, the rotting usually is confined to and may involve not more than 2 inches of one internode. The vascular bundles were observed to continue to function in some of

FIGURE 2.—Natural infection with pythium stalk rot in plants of inbred C. I. 6, yellow dent corn, grown at the Arlington Experiment Farm. Photographed August 6, 1940.

the large plants of inbred C. I. 6, so that the plants lying on the ground remained green and turgid for several days (fig. 1, *A*). Longitudinal sections of infected internodes showed water-soaked, brown tissue at the margins of infected areas and a sharp line of demarcation

FIGURE 3.—Longitudinal sections showing natural infection with pythium stalk rot in plants of inbred C. I. 6, yellow dent corn, grown at the Arlington Experiment Farm. Photographed August 6, 1940.

between the dark-brown, water-soaked tissue and healthy tissue (fig. 3). Along this line there was often a purplish or lavender tinge.

At the Arlington Experiment Farm the infection was most severe in the tender tissue of the young plants of inbred K167, which were less than 2 feet tall, and developed first just above the ground level. It extended up the stalk through two to three internodes, or to the growing point, where infected tissue was much less clearly separated from the healthy. There was no putrid odor, as with bacterial infection, but a peculiar, characteristic odor, which has been described as "marshy."

A similar stalk rot was observed early in August 1941, in a small field of Hybrid Extra Early Yellow Dent corn growing on a farm west of Petersburg, Dinwiddie County, Va. The infection was scattered through the field but was more abundant on one side, where 20 to 25 percent of the stalks had lodged. The stalks were 1½ inches in diameter, and the plants had grown vigorously and normally with no signs of disease until they were found lying on the ground. Local rotting of internodes above the brace roots, like that in the plants of inbred C. I. 6, had caused them to lodge (figs. 4 and 5).

The field was visited about 3 weeks after the infection was first reported. Some plants had died, but in others the leaves and stalks above the rotted internodes were green and turgid, roots had developed at nodes just above the rotted internodes (figs. 5 and 6), and the growing tops had turned upward, developed tassels, and in one instance an ear (fig. 4). What was left of the rotted internodes was brown and dry. There was no evidence of bacterial infection and no odor of decay. In the interior of the stalks the rotted and healthy tissues were distinctly separated. The roots were abundant and healthy.

The development of this stalk rot in Dinwiddie County, Va., in 1941, followed a period of high temperatures, as was the case in 1940, at the Arlington Experiment Farm. The temperature and rainfall data for the two localities and for the period coinciding with or immediately preceding the observations of the disease are given in table 1.

TABLE 1.—*Temperature and rainfall data for the Arlington Experiment Farm and Richmond,*[1] *Va., for the periods approximately coinciding with the appearance of the stalk rot disease of corn*

Date	Arlington Experiment Farm, 1940		Richmond, Va., 1941		Date	Arlington Experiment Farm, 1940		Richmond, Va., 1941	
	Maximum temperature	Rainfall	Maximum temperature	Rainfall		Maximum temperature	Rainfall	Maximum temperature	Rainfall
	° *F.*	*Inches*	° *F.*	*Inches*		° *F.*	*Inches*	° *F.*	*Inches*
July 15	84	0	80	0	July 24	92	3.20	86	0
16	86	0	84	0	25	96	0	89	0
17	87	0	87	.39	26	100	0	98	.48
18	93	0	88	.32	27	100	0	96	0
19	97	0	89	.62	28	102	0	98	0
20	98	0	85	.01	29	95	.05	90	0
21	99	0	84	0	30	98	.01	98	0
22	99	0	83	0	31	95	0	97	0
23	92	0	84	[2] T					

[1] The disease occurred about 5 miles west of Petersburg, Va., and 20 miles south of Richmond. Weather records for Petersburg were not available.

[2] T = trace.

THE CAUSAL ORGANISM

IDENTITY

Specimens of stalk rot from the Arlington Experiment Farm, held in the culture room in glass dishes during a hot week end, developed a mat of cottony white mycelium. Transfers were made from this mycelium, and isolations also were made from pieces of rotted tissue

FIGURE 4.—Hybrid Extra Early Yellow Dent corn near Petersburg, Dinwiddie County, Va., where 20 to 25 percent of the stalks had rotted just above the ground and fallen over early in August. Photographed September 3, 1941.

washed in sterile water, held overnight in water in the refrigerator, and plated on water agar. Several isolations were made from both K167 (culture 40–F) and C. I. 6 (culture 40–G). Pure cultures showed a typical nonseptate mycelium, which when grown on corn meal-carrot agar developed an abundance of oogonia and antheridia. Pure cultures of the fungus were identified by Charles Drechsler [4] as *Pythium butleri* Subr. The history of this species has been reviewed

[4] Pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

by Drechsler [5] under the name of *P. aphanidermatum*. In 1934 he [6] separated *P. aphanidermatum* into two species on the basis

FIGURE 5.—Stalk rot and root formation at nodes above rotted tissue on Hybrid Extra Early Yellow Dent corn, near Petersburg, Va. Photographed September 4, 1941.

of size of fruiting bodies. Zoosporangia, zoospores, and sexual structures are larger in *P. butleri* than in *P. aphanidermatum*.

[5] DRECHSLER, C. THE COTTONY LEAK OF CUCUMBERS CAUSED BY PYTHIUM APHANIDERMATUM. Jour. Agr. Res. 30: 1035–1042, illus. 1925.

[6] DRECHSLER, C. PYTHIUM BUTLERI AND P. APHANIDERMATUM. (Abstract) Phytopathology 24: 7. 1934.

The host range and distribution of the two species are still somewhat confused. Although no extensive isolations have been made to determine the distribution of *P. butleri*, it is, according to Drechsler, a widely distributed fungus in warm climates, living near the surface of the soil and destructive to a variety of hosts. He reports [7] (p. 393) that during or immediately following periods of excessively hot moist weather this fungus may become especially destructive.

Repeated attempts were made to isolate an organism from the rotted stalks collected near Petersburg, Va., that would reproduce the disease. *Pythium*, however, was not isolated and none of the other fungi isolated produced infection. *Pythium butleri* used in the same inocu-

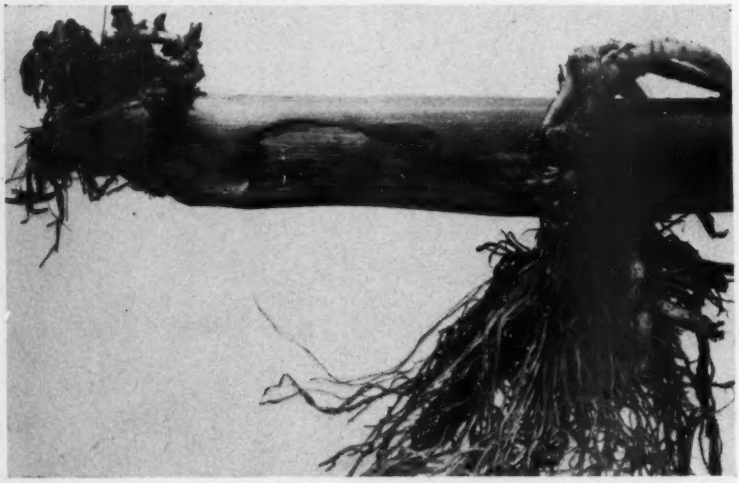

FIGURE 6.—Stalk rot, and root formation at nodes above rotted tissue, on Hybrid Extra Early Yellow Dent corn, near Petersburg, Va. Photographed September 4, 1941.

lation test produced typical stalk rot in the greenhouse at Arlington Experiment Farm. The known difficulties in isolating *Pythium* in other cases suggest that the failure to secure *Pythium* from the Petersburg corn was due to lack of fresh material and actively developing infections. The infection in the field near Petersburg, therefore, probably was due to the same fungus that caused the stalk rot in the nursery at the Arlington Experiment Farm.

PATHOGENICITY

Cultures isolated from rotted stalks of two inbred lines of yellow dent corn, K167 and C. I. 6, were tested for pathogenicity in the field and in the greenhouse at the Arlington Experiment Farm.

FIELD INOCULATIONS

Healthy plants of inbred C. I. 6, in the rows where the infection had developed, were used for inoculation with isolations from plants of K167 and C. I. 6 on August 12, 1940. The surface of the stalk

[7] DRECHSLER, C. SEVERAL SPECIES OF PYTHIUM CAUSING BLOSSOM-END ROT OF WATERMELONS. Phytopathology 29: 391–422, illus. 1939.

was washed with alcohol and mercuric chloride, an incision made, and small pieces of agar culture inserted. Three plants were inoculated with each culture. Control plants were injured in the same way but no cultures were introduced.

Maximum temperatures following inoculation were too low to be favorable for rapid development of the disease. Results of this inoculation are given in table 2, and the temperature and rainfall

TABLE 2.—*Results of inoculations in the field, August 12, 1940, of large plants of inbred C. I. 6, yellow dent corn, with cultures isolated July 30 and Aug. 3, 1940, from stalk rot lesions on K167 and C. I. 6*

Culture No.	Plants—			
	Inoculated	Fallen over		With partial stalk rot
		Aug. 16	Sept. 11	
	Number	*Number*	*Number*	*Number*
40–F–1a 1 [1]	3	0	0	3
40–F–2a [1]	3	0	0	0
40–F–3 [1]	3	0	0	3
40–G–3a 1 [2]	3	0	0	0
40–G–4c [2]	3	1	1	3
40–G–5c [2]	3	0	0	0
40–G–5b [2]	3	0	0	3
Controls	3	0	0	0

[1] Isolated from inbred K167.
[2] Isolated from inbred C. I. 6.

records for the period from August 12 to September 22, 1940, at the Arlington Experiment Farm, are given in figure 7.

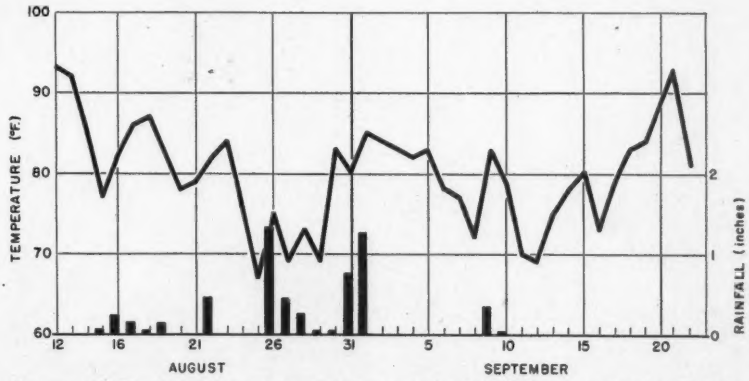

FIGURE 7.—Daily maximum temperatures and rainfall at the Arlington Experiment Farm from August 12 to September 22, 1940.

The results of an inoculation with culture 40–G–4c, which caused rotting of the inoculated internodes so that the plant fell over 4 days after inoculation, are shown in figure 8, *A*. A longitudinal section of this plant (fig. 8, *B*) shows the results of inoculations in two internodes. The infection in the lower internode progressed less rapidly than that in the upper. Some of the inoculated plants were not

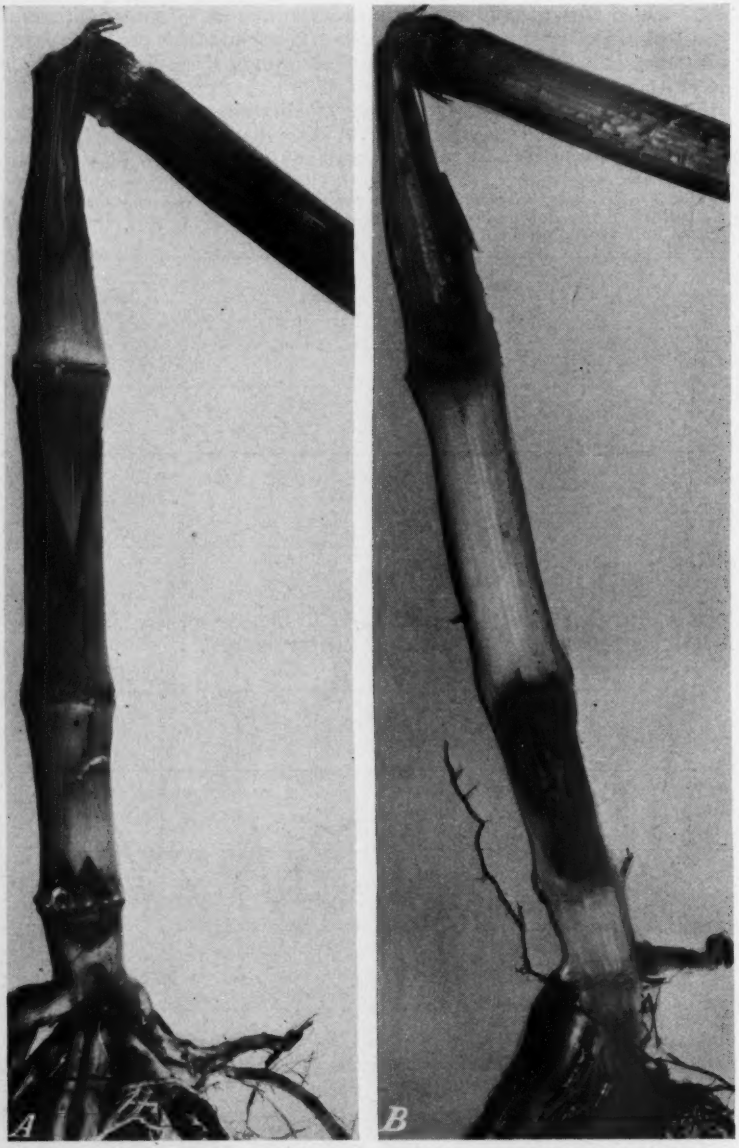

FIGURE 8.—Pythium stalk rot of C. I. 6, yellow dent corn, grown at the Arlington Experiment Farm. Plant inoculated August 12, 1940, with *Pythium butleri*, culture 40–G–4c; fallen over August 16, 1940; photographed August 24, 1940. *A*, External view; *B*, longitudinal section.

sufficiently rotted to fall over. Longitudinal sections of representative stalks of these plants are shown in figure 9. The control plant showed some discoloration around the wound (fig. 9, *A*). All four inoculated stalks showed distinct rotting of the parenchyma tissue, particularly the stalk in figure 9, *B*, which was inoculated with culture

FIGURE 9.—Pythium stalk rot on inbred C. I. 6, yellow dent corn, grown at the Arlington Experiment Farm. Inoculated August 12, 1940, with cultures of *Pythium butleri* isolated from stalk rot; photographed September 9, 1940. *A*, Wounded, uninoculated; *B–E*, inoculated with cultures 40–G–4c, 40–F–1a$_1$, 40–G–5b, and 40–F–3, respectively.

40–G–4c isolated from C. I. 6, and that in figure 9, *E*, which was inoculated with culture 40–F–3 isolated from K167. The latter stalk easily yielded to pressure, and there was little tissue left except the vascular bundles and part of the outer rind. All three plants

inoculated with culture 40–G–5b (fig. 9, *D*) showed typical water-soaked rotting and brown margins. Although the infection did not progress far enough for the plants to fall over, they were so weakened that the rind easily yielded to pressure. All three plants inoculated with culture 40–F–$1a_1$ (fig. 9, *C*) also showed typical rotting. There was no putrid odor from the infected tissue in any of these plants. *Pythium butleri* was reisolated from several of the rotted stalks.

Seed of inbred C. I. 6 was planted in the field at the Arlington Experiment Farm early in August 1940. On September 12, when the plants were about 12 inches high, inoculations were made with 13 isolations. Of six plants inoculated with each culture, two were uninjured and had the culture placed between leaf sheath and stalk, two had the stalk scratched with a needle and the culture placed between leaf sheath and stalk, and two had cultures inserted into a scalpel wound in the stalk. No infection resulted in any of these inoculated plants, apparently because environmental conditions were unfavorable. For over a week following this inoculation, daily maximum temperatures were below 90° F. and there was no rainfall.

Greenhouse Inoculations

Inoculations in the greenhouse were made (1) to determine whether *Pythium butleri* would produce a root rot of dent corn inbred lines similar to that caused by *P. arrhenomanes* as described previously,[8] (2) to obtain additional proof of the pathogenicity of the cultures of *P. butleri* isolated from the rotted stalks of dent corn described above, and (3) to test the relative susceptibility of inbred lines of dent corn to this species of *Pythium*.

In all greenhouse inoculation tests, plants were grown in sterilized white sand, to which iron as iron magnetite, 1 part in 100 by weight, was added before sterilization. The plants were grown in 6-inch pots, washed before each test for 5 minutes in 1:50 commercial formaldehyde solution, and watered with a nutrient solution, as described in a previous publication.[8] Greenhouse benches were sprayed with the formaldehyde solution. The seed was rinsed in alcohol and held for 10 minutes in a 1:1,000 mercuric chloride solution, rinsed, and germinated in Petri dishes on wet filter paper. After planting, the seedlings were allowed to grow for several days before inoculation or until they were well established.

The first test was begun November 30, 1940. In this test, the sand in 40 pots containing plants of inbred C. I. 6 was inoculated by placing centimeter squares of agar cultures near the edge of the pot, on opposite sides of the plant. Four cultures were used, 40–F–3 and 40–F–$1a_1$, isolated from inbred K167, and 40–G–5b and 40–G–4c, isolated from inbred C. I. 6. Ten pots were inoculated with each culture, and corresponding sterile agar was added to the sand in 10 control pots. Weekly measurements of height showed the same increase in inoculated and control plants. On December 16, when the sand was washed from the roots, there was no evidence of root rot. Daily maximum temperatures in the greenhouse during this period ranged from 78° to 104° F.

In May 1941, a second test for root rot was made by similar sand inoculations of 13 inbred lines of dent corn. All seedlings, after

[8] Elliott, C. Relative susceptibility to Pythium root rot of twelve dent corn inbreds. Jour. Agr. Res. 64: 711–723, illus. 1942.

being transferred from Petri dishes to sand April 26, had grown for 2 weeks on the same greenhouse bench. Daily maximum temperatures in the greenhouse at Arlington Experiment Farm during the experiment for May and June 1941 are given in figure 10.

The results agree with those of the previous test. The mean heights of the plants of all 13 inbreds on May 12, when the inoculations were made, were as follows: Uninoculated, 32.8 cm.; inoculated, 35.1 cm. At the end of the experiment the respective means were 111.2 and 118.2 cm. Rates of growth were not retarded by the inoculation, and when the sand was washed from the roots, more than 4 weeks

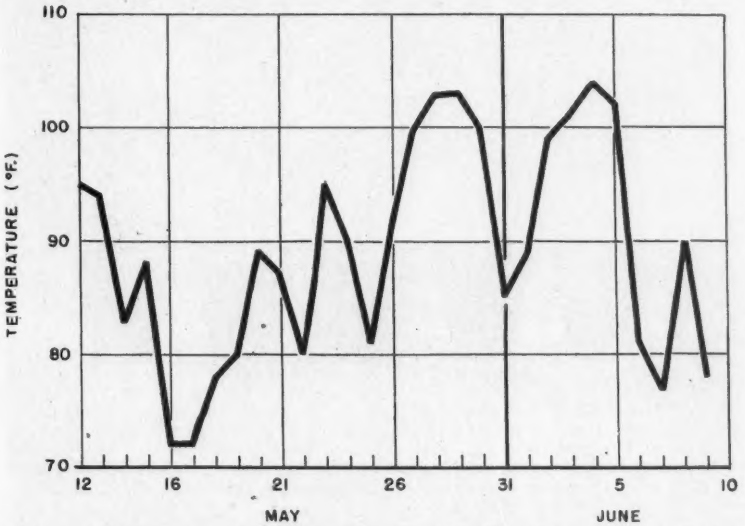

FIGURE 10.—Daily maximum temperatures in the greenhouse at the Arlington Experiment Farm during May and June 1941.

after inoculation, there was no evidence of root rot in any of the inbred lines.

Another test for root rot was made during the summer of 1941. On June 27, 13 inbred lines were grown in sand cultures and inoculated by placing cultures of 40–G–4c in the sand on opposite sides of each plant. Four pots of each were inoculated, and two of each were used as controls. Plants in inoculated sand grew as well as those in uninoculated sand, and when the sand was washed from the roots, August 4, there was no evidence of root rot. Roots in both inoculated and control plants were abundant and not discolored.

Direct inoculations of corn plants in the greenhouse to test the pathogenicity of fungus isolations from stalk rot were begun December 7, 1940. Five plants were inoculated with each of four cultures by placing pieces of agar cultures inside the lower leaf sheath. So far as could be observed, the plants had not been wounded. The results of this inoculation are given in table 3. Daily maximum temperatures in the greenhouse at Arlington Experiment Farm during the experiment, December 7 to 17, were 82°, 98°, 94°, 93°,

90°, 96°, 104°, 90°, 94°, 100°, and 97° F., respectively. Four days after inoculation, three plants inoculated with culture 40–F–3 developed stalk rot near the point of inoculation and fell over. One plant inoculated with culture 40–F–$1a_1$ had fallen over. Five days after inoculation, all five plants inoculated with culture 40–F–3 and two inoculated with each of the other three cultures had rotted and fallen. None of the five controls became infected.

Figure 11 shows the results of the test just described. Apparently *Pythium butleri* is able to penetrate unwounded stalk tissue.

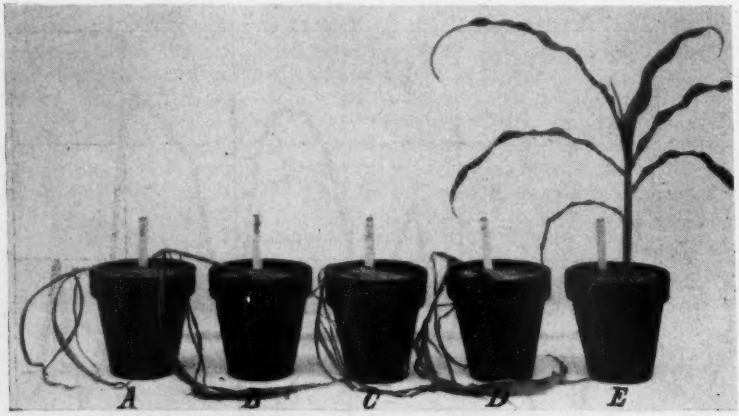

FIGURE 11.—Stalk rot of inbred C. I. 6, yellow dent corn, grown in sand plus nutrient solution in the greenhouse at the Arlington Experiment Farm. Inoculated December 7, 1940, with *Pythium butleri*, cultures (*A*) 40–F–3, (*B*) 40–G–5b, (*C*) 40–F–$1a_1$, and (*D*) 40–G–4c, respectively, isolated from rotted stalks. *E*, Uninoculated control. Photographed December 12, 1940.

TABLE 3.—*Results of greenhouse inoculations of inbred C. I. 6, yellow dent corn, grown in sand plus nutrient solution, with cultures of Pythium butleri isolated from stalk rot at Arlington Experiment Farm*

[Inoculations made without wounding plants, December 7, 1940]

Culture No.	Plants—		
	Inoculated	Fallen over—	
		Dec. 11	Dec. 12
	Number	*Number*	*Number*
40–F–3	5	3	5
40–G–5b	5	0	2
40–F–$1a_1$	5	1	2
40–G–4c	5	0	2
Controls	5	0	0

On June 6, 1941, 13 inbred lines of dent corn were inoculated to test the pathogenicity of culture 40–G–4c and to obtain information on possible differences in susceptibility to attack by *Pythium butleri*. Five plants of each inbred were inoculated by inserting mycelium into the stalk 1 to 2 inches above the soil line. Similar incisions were

made in 5 control plants of each inbred. The results are given in figure 12, *A* and *B*, and in table 4.

Maximum greenhouse temperatures for June 6, 7, 8, and 9 were 98°, 96°, 97°, and 94° F., respectively, and the humidity was high. On June 9, 3 days after inoculation, all but 8 of the 65 inoculated

FIGURE 12.—Thirteen inbred lines of dent corn, grown in sand plus nutrient solution; planted April 26, 1941. *A*, Stalks wounded at base June 6, 1941, but not inoculated; photographed June 9, 1941. *B*, Plant inoculated June 6, 1941, by wounding and inserting *Pythium butleri* into lower internode of stalk; photographed June 9, 1941. (Plants at farther end of bench were not inoculated.)

plants had fallen over. The stalk tissue was water-soaked and rotted for 1 to 2 inches around the point of inoculation. The attendant who watered the plants over the week end said they were lying flat on the bench on Sunday, only 48 hours after inoculation. All the inoculated plants were cut lengthwise to determine the extent of

rotting. Only 1 inbred, Ill. Hy., showed any marked resistance. In this line, only 1 plant in 5 fell over and rotting around the point of inoculation was very limited. In Ky. 13, 3 out of 5 plants fell over, and likewise 4 out of 5 plants of Ill. R4 and Ia. L317. Five inbreds were very susceptible.

TABLE 4.—*Inbred lines of dent corn inoculated June 6, 1941, with Pythium butleri, culture 40–G–4c, to determine differences in susceptibility to stalk rot*

Inbred—	Plants—			Reaction to inoculation
	Inoculated	Fallen over June 9	With stalk rot	
	Number	*Number*	*Percent*	
C. I. 6:				
Inoculated	5	5	100	Very susceptible.[1]
Control	5	0	0	
Ill. Hy.:				
Inoculated	5	1	20	Resistant.
Control	5	0	0	
C. I. 540:				
Inoculated	5	5	100	Susceptible.
Control	5	0	0	
C. I. 5:				
Inoculated	5	5	100	Moderately susceptible.
Control	5	0	0	
Ia. L289:				
Inoculated	5	5	100	Very susceptible.
Control	5	0	0	
Ia. Mc401:				
Inoculated	5	5	100	Very susceptible.
Control	5	0	0	
Ky. 13:				
Inoculated	5	3	60	Moderately susceptible.
Control	5	0	0	
C. I. 1:				
Inoculated	5	5	100	Very susceptible.
Control	5	0	0	
Ill. R4:				
Inoculated	5	4	80	Moderately susceptible.
Control	5	0	0	
C. I. 4–8:				
Inoculated	5	5	100	Susceptible.
Control	5	0	0	
Ia. L317:				
Inoculated	5	4	80	Moderately susceptible.
Control	5	0	0	
Ind. Tr.:				
Inoculated	5	5	100	Very susceptible.
Control	5	0	0	
Ia. B1 345:				
Inoculated	5	5	100	Susceptible.
Control	5	0	0	

[1] Very susceptible=rot spreading throughout the stalk and into the growing point; no sharp line of demarcation between rotted and healthy tissue. Susceptible=rot spreading beyond inoculated internode, but sharp line between infected and healthy tissue. Moderately susceptible=rotting mostly confined to inoculated internode. Resistant=very limited rotting around point of inoculation.

On July 29, 1941, a limited number of plants of the same 13 inbreds were inoculated with culture 40–G–4c, to test further differences in susceptibility and to test again the ability of the fungus to infect uninjured as well as injured stalk tissue. This experiment was discontinued August 4, 6 days after the inoculations were made. The results are given in table 5.

Daily maximum greenhouse temperatures during the period July 29 to August 4, 1941, were 91°, 96°, 100°, 100°, 100°, 102°, and 102° F., respectively. In spite of these temperatures, conditions apparently were less favorable for infection than in the previous test; infection developed less rapidly and differences between inbreds were somewhat more apparent. Ill. Hy. and Ky. 13 again were the most resistant inbreds. While none of the plants of C. I. 6 fell over, sections of

inoculated stalks showed definite internal rotting. Infection developed most rapidly in C. I. 540, C. I. 5, Ia. L289, and C. I. 1; C. I. 4–8 and Ind. Tr. were almost as susceptible. Infection again developed without wounding.

TABLE 5.—*Results of greenhouse inoculations of 13 inbred lines of dent corn with Pythium butleri, culture 40–G–4c, July 29, 1941, Arlington Experiment Farm*

Inbred and method of inoculation [1]	Plants—				Remarks
	Inoculated	Rotted and fallen over—			
		3d day	4th day	6th day	
C. I. 6:					
Inoculated:	*Number*	*Number*	*Number*	*Number*	
Wounded	1	0	0	0	Internode water-soaked inside.
Not wounded	3	0	0	0	
Control	2	0	0	0	
Ill. Hy.:					
Inoculated:					
Wounded	2	0	0	0	Brown rot ½ inch around inoculation.
Not wounded	3	0	0	0	
Control	1	0	0	0	
C. I. 540:					
Inoculated:					
Wounded	1	0	1	1	
Not wounded	3	1	1	1	
Control	2	0	0	0	
C. I. 5:					
Inoculated:					
Wounded	1	1	1	1	
Not wounded	3	0	0	0	
Control	2	0	0	0	
Ia. L289:					
Inoculated:					
Wounded	1	1	1	1	
Not wounded	3	0	0	1	
Control	2	0	0	0	
Ia. Mc401:					
Inoculated:					
Wounded	1	0	0	1	
Not wounded	3	0	0	0	
Control	2	0	0	0	
Ky. 13:					
Inoculated:					
Wounded	1	0	0	0	Brown rot ½ inch around inoculation.
Not wounded	3	0	0	0	
Control	2	0	0	0	
C. I. 1:					
Inoculated:					
Wounded	1	1	1	1	
Not wounded	3	0	0	0	
Control	2	0	0	0	
Ill. R4:					
Inoculated:					
Wounded	1	0	0	1	
Not wounded	3	0	0	0	
Control	2	0	0	0	
C. I. 4–8:					
Inoculated:					
Wounded	1	0	1	1	
Not wounded	3	0	0	0	
Control	2	0	0	0	
Ia. L317:					
Inoculated:					
Wounded	1	0	0	0	Rotted 1 inch around inoculation.
Not wounded	3	0	0	1	
Control	2	0	0	0	
Ind. Tr.:					
Inoculated:					
Wounded	1	0	1	1	
Not wounded	3	0	0	0	
Control	2	0	0	0	
Ia. B1 345:					
Inoculated:					
Wounded	1	0	0	0	
Not wounded	2	0	0	1	
Control	0	0	0	0	

[1] In each case the wounded inoculated plants had mycelium introduced into an incision in their stalks, and the unwounded ones had it placed between the stalk and lower leaf sheath.

On September 9, 1941, *Pythium butleri*, culture 40–G–4c, isolated originally from inbred C. I. 6, was used to inoculate cucumber (*Cucumis sativus* L.), and three kinds of squash (*Cucurbita pepo* L.), namely, crookneck, Patty Pan, and Vegetable Marrow, in evaporating dishes in the laboratory. The two latter had very young tender tissue; the two former, rather old and hard tissue. Incisions were made in each specimen and pieces of mycelium were introduced

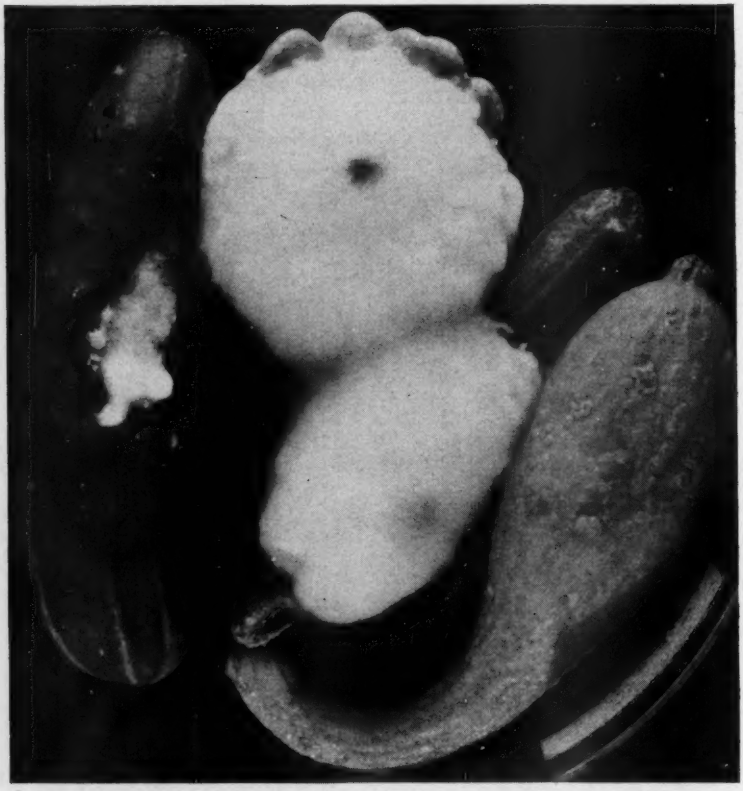

FIGURE 13.—*Pythium butleri*, culture 40–G–4c, on cucumber, Patty Pan squash crookneck squash, and Vegetable Marrow. Inoculated September 9, 1941; photographed September 11, 1941. September 15, 1941, all four were covered with an abundant mass of white mycelium.

Maximum temperatures in the laboratory on September 9 and 10 were 95° and 96° F. and the humidity was high. After 24 hours both Patty Pan squash and Vegetable Marrow showed water-soaked areas around the point of inoculation. Two days after inoculation these two squashes were almost covered with a cottony white mass of mycelium (fig. 13) similar to that described and illustrated by Drechsler [9] for this fungus. A white mycelium had broken out in several places for 2 inches around the inoculation on cucumber. Six days

[9] See footnote 5.

after inoculation, all four specimens were practically covered with an abundant growth of white mycelium.

CONTROL

Inasmuch as the disease developed only on certain corn inbreds in the nursery and not on inbreds in alternating rows, and as inoculations have shown that some inbreds are more resistant to infection than others, it seems that the best means of control is through the use of resistant strains.

Because of the exacting thermal requirement of *Pythium butleri*, it seems probable that this stalk rot may be of importance only in the southern parts of the Corn Belt.

SUMMARY

Stalk rot of two inbred lines (K167 and C. I. 6) of yellow dent corn (*Zea mays*) was observed in the field at the Arlington Experiment Farm in late July and early August 1940. The rotting in the larger plants was limited to one or more of the internodes above the brace roots. Infected plants fell over but remained green and turgid for several days. The infection became apparent after a period of hot humid weather. Successful inoculations with cultures isolated from this stalk rot were made during periods of high temperature and high humidity, but inoculations made when the temperature was lower produced little or no infection.

Pythium butleri Subr. was isolated from the rotted internodes and the disease was reproduced on inbred C. I. 6 in the field and in the greenhouse by inoculations with this fungus. Infections developed on both wounded and unwounded inoculated stalks.

Greenhouse inoculations on inbred C. I. 6 and on 12 other inbred lines of dent corn showed that some lines were more resistant than others. C. I. 5, Ia. L289, C. I. 1, and C. I. 540 were susceptible, and Ill. Hy. and Ky. 13 were resistant.

In greenhouse inoculations, *Pythium butleri* did not attack the corn roots.

Cultures of *Pythium butleri* isolated from rotted cornstalks rapidly rotted squashes and cucumber and covered them with a cottony mass of white mycelium, typical of this fungus.

What appeared to be the same disease occurred in a commercial field of hybrid corn near Petersburg, Va., August 1941.

FURTHER STUDIES ON INTERSPECIFIC GENETIC RELATIONSHIPS IN LACTUCA[1]

By Ross C. Thompson[2]

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INTRODUCTION

In an earlier paper, Thompson, Whitaker, and Kosar[3] reported on some genetic relationships between certain species of the 9-chromosome and of the 17-chromosome group of the genus *Lactuca*. Whitaker and Thompson[4] presented data on some cytological aspects of these same species. In the first of these papers mention was made of 2 of the 8-chromosome species, but no compatibility relationships were presented. The present paper shows some of the compatibility relationships found to exist between certain of the 8-chromosome species and species of the 9- and 17-chromosome groups. The studies on species relationships were undertaken for the purpose of isolating genetic factors for resistance to certain lettuce diseases, aster yellows in particular, and factors for hardiness and of determining the extent to which the species carrying such factors can be crossed with the cultivated form *Lactuca sativa*. This paper concludes certain phases of these studies and summarizes the contributions made to date.

MATERIALS AND METHODS

The original seed of the species used in the hybridization studies reported were obtained from the following sources:

Eight-chromosome species:

Lactuca bourgaei (Boiss.) Irish and Taylor, from England, through Division of Plant Exploration and Introduction.
L. marschallii Stebbins, from Sweden, through G. L. Stebbins, Jr.
L. cretica Desf., from Africa, through G. L. Stebbins, Jr.
L. capensis var. *duruensis* De Wild., from Belgian Congo, through G. L. Stebbins, Jr.

[1] Received for publication May 21, 1942.

[2] The writer is indebted to G. L. Stebbins, Jr., Department of Genetics, University of California, for seed and for identification of some of the species; to S. F. Blake, of the Division of Plant Exploration and Introduction, Bureau of Plant Industry, for identification of some of the species and for verification of the botanical names; to the Division of Plant Exploration and Introduction for supplying seed of some of the foreign species; and to T. W. Whitaker, U. S. Department of Agriculture Horticultural Field Station, La Jolla, Calif., for the results presented in table 1 on the attempted crosses between the 8-chromosome species *Lactuca capensis* and 5 species representing the 8-, 9-, and 17-chromosome groups.

[3] Thompson, R. C., Whitaker, T. W., and Kosar, W. F. Interspecific genetic relationships in Lactuca. Jour. Agr. Res. 63:91–107, illus. 1941.

[4] Whitaker, T. W., and Thompson, R. C. Cytological studies in Lactuca. Torrey Bot. Club Bul. 68:388–394, illus. 1941.

Nine-chromosome species:

L. tatarica (L.) C. A. Mey., from Cap d'Antibes, France, through G. L. Stebbins, Jr.
L. indica L., from China, through Division of Plant Exploration and Introduction.
L. raddeana Maxim., from Union of Soviet Socialist Republics, through Division of Plant Exploration and Introduction.
L. squarrosa (Thunb.) Miq.,[5] from Asia, through Division of Plant Exploration and Introduction.
L. perennis L., from Wayside Gardens, Mentor, Ohio.
L. serriola L., collected in District of Columbia.
L. saligna L., from Ohio, through G. L. Stebbins, Jr.
L. virosa L., from Surrey, England, through Division of Plant Exploration and Introduction.
L. sativa L., from private stocks.

Seventeen-chromosome species:

L. canadensis L., collected in Maryland.
L. spicata (Lam.) Hitchc., collected in Virginia.
L. floridana (L.) Gaertn., collected in Maryland.
L. graminifolia Michx., from South Carolina, through the late J. B. Norton.

All the parent and hybrid plants were grown in 10-inch clay pots under partly controlled conditions in a closely screened greenhouse at Beltsville, Md.

Except in the case of *Lactuca tatarica*, which was entirely self-sterile under the conditions prevailing in the greenhouse, flower heads of the maternal parents were depollinated with water. Pollen from the desired male parent was transferred by touching the pollen-laden stigmas and styles to the washed stigmas of the female flowers. Ten or more flower heads were pollinated in each attempted cross.

RESULTS

The results obtained from crosses attempted between the 8-chromosome species and species of the 8-, 9-, and 17-chromosome groups are presented in table 1.

TABLE 1.—*Summary of data on interspecific hybridization of some 8-chromosome species with certain 8-, 9-, and 17-chromosome species of Lactuca* [1]

Species and number of chromosomes	Results of cross		Fertility of F_1	
	Cross as indicated	Reciprocal	Cross as indicated	Reciprocal
L. bourgaei (8) ×:				
L. tatarica (9)	*O*	*T*		*S*
L. indica (9)	*O*	*O*		
L. squarrosa (9)	*O*	*O*		
L. raddeana (9)	*O*	*O*		
L. perennis (9)	*O*	*N*		
L. serriola (9)	*O*	*O*		
L. virosa (9)	*O*	*O*		
L. saligna (9)	*N*	*O*		
L. sativa (9)	*O*	*O*		
L. canadensis (17)	*N*	*T*		*S*
L. spicata (17)	*N*	*T*		*U*
L. graminifolia (17)	*N*	*T*		*S*
L. floridana (17)	*N*	*O*		
L. marschallii (8)	*T*	*T*	*F*	*F*
L. cretica (8)	*O*	*O*		

See footnotes at end of table.

[5] Called *Lactuca laciniata* in the paper cited in footnote 3. The writer has been informed by S. F. Blake and G. L. Stebbins, Jr., that the species formerly called *L. laciniata* should be classified as *L. squarrosa*.

TABLE 1.—*Summary of data on interspecific hybridization of some 8-chromosome species with certain 8-, 9-, and 17-chromosome species of Lactuca* [1]—Continued

Species and number of chromosomes	Results of cross		Fertility of F_1	
	Cross as indicated	Reciprocal	Cross as indicated	Reciprocal
L. marschallii (8) ×:				
L. indica (9)	*O*	*O*		
L. squarrosa (9)	*O*	*O*		
L. raddeana (9)	*O*	*O*		
L. perennis (9)	*O*	*N*		
L. serriola (9)	*O*	*O*		
L. virosa (9)	*O*	*O*		
L. saligna (9)	*O*	*O*		
L. sativa (9)	*O*	*O*		
L. canadensis (17)	*O*	*N*		
L. graminifolia (17)	*N*	*T*		*S*
L. floridana (17)	*N*	*T*		*S*
L. cretica (8) ×:				
L. tatarica (9)	*T*	*T*	*S*	*S*
L. indica (9)	*O*	*O*		
L. squarrosa (?)	*O*	*O*		
L. raddeana (9)	*O*	*O*		
L. perennis (9)	*O*	*O*		
L. serriola (9)	*O*	*O*		
L. virosa (9)	*O*	*O*		
L. saligna (9)	*O*	*O*		
L. sativa (9)	*O*	*O*		
L. canadensis (17)	*O*	*N*		
L. graminifolia (17)	*O*	*N*		
L. floridana (17)	*O*	*N*		
L. spicata (17)	*O*	*N*		
L. capensis (8) ×: [2]				
L. cretica (8)	*O*	*N*		
L. sativa (9)	*O*	*N*		
L. virosa (9)	*O*	*N*		
L. perennis (?)	*O*	*N*		
L. graminifolia (17)	*O*	*N*		

[1] Key to letter symbols:
F, the hybrids obtained were self-fertile.
N, the cross was not attempted.
O, the cross was attempted but failed.
S, hybrids obtained were self-sterile.
T, hybrid seedlings were obtained.
U, the fertility of this hybrid is not yet known.

[2] Data furnished by T. W. Whitaker.

In the investigations reported herein, 69 crosses were attempted between the 8-chromosome species and species with 8, 9, or 17 chromosomes; 25 of these were reciprocal crosses. Hybrids were obtained in only 8 of the 44 interspecific matings. All of these hybrids were self-sterile except those from the cross between the two 8-chromosome species, *Lactuca bourgaei* × *L. marschallii*, which were highly fertile, and the one from *L. spicata* × *L. bourgaei*, which had not produced flowers 9 months after germination. In 2 cases the hybrids obtained were from crosses between 8- and 9-chromosome species and in 5 cases from crosses between 8- and 17-chromosome species. Although in a few cases reciprocal hybrids were obtained, study was made only on plants from 1 group, as indicated by the order in which the species names are given in the discussion of each hybrid.

CROSSES BETWEEN EIGHT-CHROMOSOME SPECIES

Lactuca bourgaei (8) × *L. marschallii* (8) (fig. 1, *A*).—Reciprocal crosses between these two species were easily obtained, and fertile progenies resulted in both cases. The similarity of these two forms in many of their morphological characters combined with their breeding behavior indicates that they should be considered as varieties of the same species.

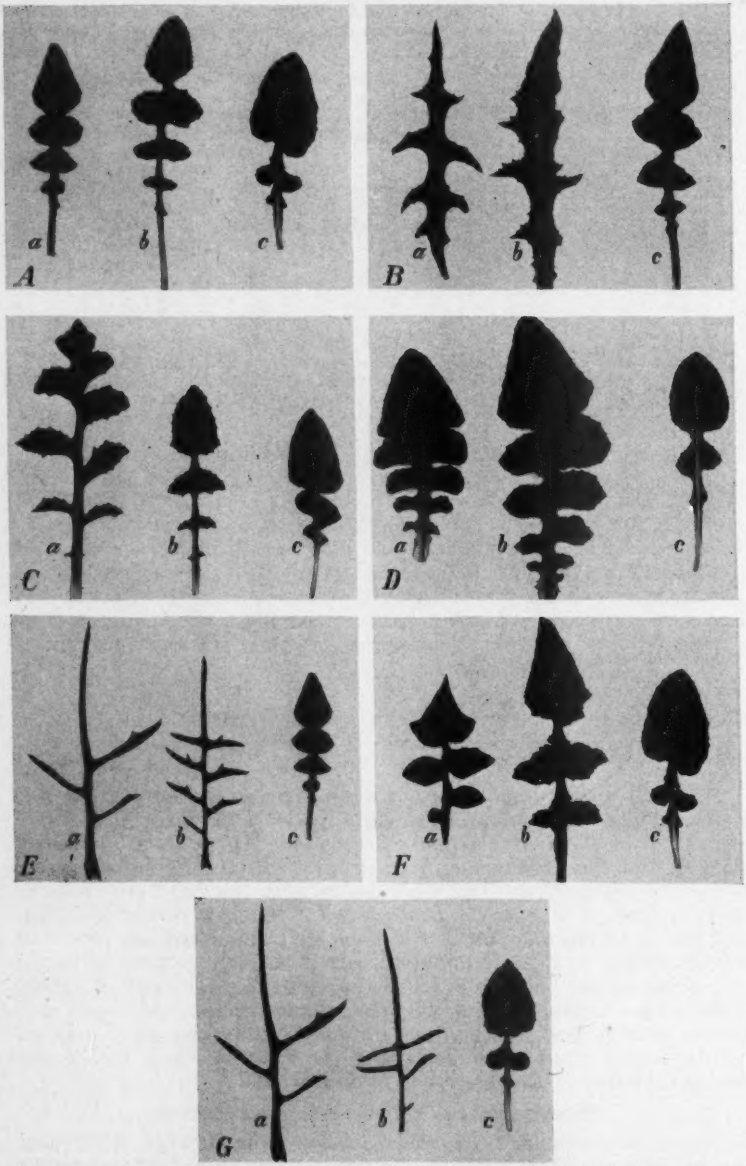

FIGURE 1.—Representative rosette leaves of various F_1 *Lactuca* hybrids and their parents. In each group *a* indicates the female parent, *b* the hybrid, and *c* the male parent. *A*, *L. bourgaei* × *L. marschallii*; *B*, *L. atarica* × *L. bourgaei*; *C*, *L. canadensis* × *L. bourgaei*; *D*, *L. spicata* × *L. bourgaei*; *E*, *L. graminifolia* × *L. bourgaei*; *F*, *L. floridana* × *L. marschallii*; *G*, *L. graminifolia* × *L. marschallii*.

CROSSES BETWEEN EIGHT- AND NINE-CHROMOSOME SPECIES

Lactuca tatarica (9) × *L. bourgaei* (8) (fig. 1, *B*).—Hybrid seed was easily obtained from these 2 species when pollen of *L. bourgaei* was applied to the heads of *L. tatarica*. A population of 25 F_1 plants was grown, and all were completely self-sterile. There were some minor morphological variations among the F_1 plants.

The hybrid plants were in many respects intermediate but in general appearance resembled *Lactuca tatarica* much more than *L. bourgaei*. They did not exhibit marked hybrid vigor. Most of them attained a greater height than either parent but were not extremely large in any of their characters. All of them sprouted from the base and from the roots as does *L. tatarica*. The flower heads were a little larger than those of either parent; the corolla color was a light blue, intermediate between those of the two parents, both of which have blue flowers.

Lactuca tatarica (9) × *L. cretica* (8).—From seed developing from flowers of *L. tatarica* pollinated with pollen from *L. cretica* were obtained 17 hybrid plants, all of which were entirely self-sterile. In general appearance the hybrids resembled *L. cretica* more than *L. tatarica*. The leaves were smaller than those of *L. tatarica*, and the plants were very short-stemmed like those of *L. cretica*. None of the hybrid plants reached a height of more than a foot, and all showed marked lack of vigor. The flowers were larger than those of *L. tatarica* but did not develop the large robust heads characteristic of *L. cretica*. The spread of the corolla approximated that of *L. cretica*. The petals were a very light shade of blue, much lighter than is typical for *L. tatarica*. *L. cretica* has yellow flowers.

CROSSES BETWEEN 8- AND 17-CHROMOSOME SPECIES

Lactuca canadensis (17) × *L. bourgaei* (8) (fig. 1, *C*).—Several flower heads of *L. canadensis* were pollinated with pollen from *L. bourgaei*. Seven hybrid plants were obtained from the seed resulting from the cross pollinations, and all seven were entirely self-sterile.

The hybrid plants were intermediate between the two parents in many respects but in general appearance resembled small plants of *Lactuca canadensis* more than *L. bourgaei*. The hybrids lacked vigor, but all seven of them developed through the flowering stage. The plants reached about the same height (about 5 feet) as plants of *L. bourgaei* growing in the same greenhouse. Plants of *L. canadensis* in the same house reached a height approximately twice that of *L. bourgaei* and of the hybrids.

The flowers of the hybrids were a little larger than those of *Lactuca canadensis* and about the same shade of yellow. The yellow color of the corolla of these hybrids is contrary to expectation, since in all other interspecific hybrids studied yellow corolla color has been recessive to any shade of blue. These two species differ in many morphological characters, and distinct characteristics of both species were discernible in the hybrids. However, there is an element of doubt in regard to these hybrids.

Lactuca spicata (17) × *L. bourgaei* (8) (fig. 1, *D*).—Crosses between these two species were difficult to effect. Nine plants grown from seed obtained by applying pollen of *L. bourgaei* to washed flower heads of *L. spicata* consisted of eight selfed *L. spicata* and one large vigorous hybrid. In the rosette stage the hybrid plant showed marked

hybrid vigor. This plant seemed to have a biennial or perennial habit, as it failed to develop a seed stem during the first season; 9 months after the seed was germinated there was no evidence of stem elongation.

Lactuca graminifolia (17) × *L. bourgaei* (8) (fig. 1, *E*).—From flower heads of *L. graminifolia* pollinated with pollen from *L. bourgaei*, 31 seeds were obtained. In a population of F_1 plants from the 31 seeds there were 13 that were obviously selfed and 13 hybrids, of which 11 appeared to be normal and 2 were weak abnormal plants that died in the seedling stage. Five seeds failed to germinate. The 11 hybrid plants that appeared to be normal were grown to maturity, and all were completely self-sterile.

The hybrid plants showed some hybrid vigor, reaching a height of about 7 feet, while the *Lactuca graminifolia* plants in the same environment averaged about 2½ feet and the *L. bourgaei* plants about 4 feet. In general appearance the hybrids resembled *L. graminifolia* much more than *L. bourgaei*. In size the flowers were intermediate between those of the two parent species. The color of the corollas was a shade of bluish purple, which is intermediate between the corolla colors of the two parents.

Lactuca floridana (17) × *L. marschallii* (8) (fig. 1, *F*).—Five seeds were obtained from flower heads of *L. floridana* to which pollen of *L. marschallii* was applied. All but one of these proved to be selfed *L. floridana*. The one hybrid was a fairly vigorous plant that reached a height of 5½ feet at maturity. In general appearance it resembled *L. floridana*. Plants of *L. floridana* reached a height of about 7 feet and those of *L. marschallii* about 4 feet in the same environment in which the hybrid was grown. The flowers were intermediate in size, and the color of the corolla was blue, intermediate between those of the parents. The hybrid was completely self-sterile.

Lactuca graminifolia (17) × *L. marschallii* (8) (fig. 1, *G*).—Crosses between these 2 species were easily effected when pollen of *L. marschallii* was applied to washed stigmas of *L. graminifolia*. Thirty-two normal hybrids were obtained from an F_1 population of 44 plants. There were no weak freakish hybrids from this cross. Eleven of the 32 hybrids were grown to maturity, and all were completely self-sterile.

The hybrids were quite uniform in appearance and resembled the *Lactuca graminifolia* parent more than *L. marschallii*. These plants reached an average height of about 7 feet as compared with 4 feet for *L. marschallii* and 2½ feet for *L. graminifolia* plants in the same environment. The flowers were intermediate in size. The corollas were a bluish-purple, intermediate between those of the two parents, both of which are blue-flowered.

DISCUSSION

One of the contributions of the present study is the revelation of the compatibility of the chromosome complements of certain of the 8-chromosome species with the complements of at least 1 of the 9-chromosome species and of 4 of the 17-chromosome species of *Lactuca*. Probably the most important fact demonstrated is the compatibility relationship shown to exist between the 9-chromosome species *L. tatarica*, a member of the *indica* group, and the two 8-chromosome

species *L. bourgaei* and *L. cretica*. These last 2 species are generally classified taxonomically in separate subgenera, *Mulgedium* and *Serriola*, respectively.

Although the F_1 hybrids of *Lactuca tatarica* × *L. bourgaei* and *L. tatarica* × *L. cretica* were in both cases completely self-sterile, the development of F_1 hybrids that grew through to flower production as quite normal plants shows that the genoms of at least some of the eight- and nine-chromosome species of *Lactuca* are compatible enough to function harmoniously throughout somatic development.

It has been reported [6] that the 9-chromosome *Lactuca tatarica* differs from the 8-chromosome forms *L. bourgaei* and *L. marschallii* chiefly in the addition of a small pair of medianly constricted chromosomes. Root-tip smears of both of the hybrids showed 17 somatic chromosomes. This is substantial evidence in support of the generally accepted hypothesis that the 17 chromosome species of the genus *Lactuca* had their genesis in hybrids between 8- and 9-chromosome forms.

If some condition should cause chromosome doubling in either of these 2 hybrids, an artificial 17-chromosome form would result. It is not suggested that the species employed in obtaining the 2 above-mentioned hybrids are necessarily the forms that may have entered into the origin of the present 17-chromosome species. Neither of the hybrids obtained between 8- and 9-chromosome species closely resemble any of the 17-chromosome species with which the writer is acquainted. Although the species used are probably not the ones most likely to have entered into the origin of the 17-chromosome species, the compatibility shown by the development of normal F_1 hybrid plants indicates the possibility of the 17-chromosome forms, having come into existence through such hybrids.

In the previous paper [7] it was shown that compatibility exists between certain of the 9-chromosome species of the *indica* group and some of the 17-chromosome species. The results of the present studies show that compatibility relationship also exists between some of the 8-chromosome species and these same 17-chromosome forms, *Lactuca canadensis*, *L. spicata*, *L. graminifolia*, and *L. floridana*. Normal hybrid plants were obtained from crosses of *L. bourgaei* with *L. canadensis*, *L. spicata*, and *L. graminifolia* and from crosses of *L. marschallii* with *L. graminifolia* and *L. floridana*. In all of the 5 crosses the 17-chromosome form was used as the maternal parent.

In the previous paper [7] were presented data that showed a close relationship between the 4 above-named 17-chromosome species. Additional data have been recorded since that report. Partly fertile lines have been obtained from the following crosses: *Lactuca canadensis* × *L. graminifolia*, *L. canadensis* × *L. spicata*, *L. canadensis* × *L. floridana*, *L. graminifolia* × *L. floridana*, and a 3-species hybrid in which an F_3 selection from the *L. canadensis* × *L. floridana* hybrids was crossed onto *L. graminifolia*. The first 2 and the last of these 5 hybrids are almost completely self-fertile. The other 2 are only partly fertile. These results indicate a closer relationship between these 4 species than their taxonomic classification might suggest,

[6] Babcock, E. B., Stebbins, G. L., Jr., and Jenkins, J. A. Chromosomes and phylogeny in genera of the Crepidineae. Cytologia Fujii Jubilaei Volumen, pt. 1, pp. 188-210, illus. 1937.

[7] See footnote 3.

since *L. canadensis* and *L. graminifolia* are placed in the subgenus *Serriola* and *L. spicata* and *L. floridana* in the subgenus *Mulgedium*.

Many attempts to cross the eight-chromosome species *Lactuca bourgaei*, *L. marschallii*, and *L. cretica* with the nine-chromosome species of the *serriola* group failed. The results obtained indicate that these three eight-chromosome forms are more closely related to the nine-chromosome species of the *indica* group than to the nine chromosome species of the *serriola* group.

The data presented in this and the previous paper support the following conclusions: The serriolalike 9-chromosome species *Lactuca serriola*, *L. virosa*, *L. altaica*, *L. saligna*, *L. sativa*, and closely related species constitute a compatibility group not closely related genetically to the 9-chromosome species of the *indica* group, *L. indica*, *L. squarrosa*, *L. tatarica*, and *L. raddeana*, or to any of the 8- and 17-chromosome species studied. This group of species seems to stand apart from the other groups of species. The species of the *indica* group listed above form a compatibility group among themselves and show compatibility with some of the 8- and 17-chromosome species studied.

The results of these matings show that the genoms of the 8-chromosome *Lactuca bourgaei* and the 9-chromosome *L. tatarica* are compatible to the extent of acting harmoniously together in the somatic development of hybrids between the 2 species. Moreover the genoms of each of these 2 species are capable of existing harmoniously in the somatic development of hybrids between each of them and at least three of the four 17-chromosome species studied, *L. canadensis*, *L. spicata*, and *L. graminifolia*. *L. tatarica* was also successfully mated with *L. floridana*.

No hybrids were obtained from any of the attempted crosses between the 8-chromosome species *Lactuca capensis* and species of the 8-, 9-, and 17-chromosome groups.

SUMMARY

The results are presented from 69 attempted crosses between 8-, 9-, and 17-chromosome species of *Lactuca;* 25 of these were reciprocal crosses.

Only 8 of the 44 one-way matings resulted in hybrids and only 1 of these *Lactuca bourgaei* × *L. marschallii* was fertile. No fertile hybrids were obtained from matings of 8-chromosome species with species of a different chromosome number, with the possible exception of *L. spicata* × *L. bourgaei*.

Somatic compatibility was found to exist between the chromosome complements of the 8-chromosome species when crossed with certain 9-chromosome and with certain 17-chromosome species.

Further evidence is presented to show that the *serriola* group, to which the cultivated form *Lactuca sativa* belongs, constitutes a compatibility group not closely related to the other groups of species studied.

Successful crosses between certain 8- and 9-chromosome species, although resulting in sterile hybrids, present further evidence in support of the hypothesis that the 17-chromosome species have come into existence through hybrids between 8- and 9-chromosome species.

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